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TWO HYPHOMYCETES PARASITIC ON OOSPORES OF ROOT-ROTTING OOMYCETES

CHARLES DRECHSLER

(Accepted for publication Sept. 18, 1937)

When sizeable masses of tissue in the larger roots, as also in the basal portions of the stems of plants—more especially of herbaceous plants—are invaded and killed by phycomycetous parasites of the genera *Pythium*, *Phytophthora*, and *Aphanomyces*, they usually come to be permeated by an assortment of adventitious organisms wherein bacteria, fungi, protozoa, and nematodes are represented in greater or smaller measure. As these intrusive organisms cannot well be reached with surface disinfectants, isolation of the oomycetous pathogens primarily responsible for root rots needs to be accomplished in almost all instances through procedure that, except for some discouragement of bacterial multiplication at the beginning, cannot well aim at suppression of such forms of life as may be present (7). Accordingly, after a week or two, the isolation cultures started from the affected tissues often show luxuriant development of microscopic plants and animals, some of which then conveniently reveal on the transparent substratum characteristic parasitic, predacious, or other biotic relationships that undoubtedly prevail also on the natural substrata, but there ordinarily remain concealed from view because of the opaqueness of these materials.

OCCURRENCE, MORPHOLOGY, AND CULTURAL CHARACTERS OF DACTYLELLA SPERMATOPHAGA

Among the organisms thus appearing time after time, year in, year out, in isolation cultures of many species of *Pythium*, is a moniliaceous fungus that I first took for a type of *Fusarium* differing from the general run of forms referred to that genus in its slow growth and in the scanty production of its conidia singly on erect aerial conidiophores. It was noticed early that here and there a branch of the septate mycelium passed narrowly through the wall of an oospore, conidium, or zoosporangium belonging to the root-rotting phycomycete cultured, and, within the invaded reproductive body, terminated in swollen lobulate elements. Though the swollen elements clearly had the appearance of assimilative structures, their presence in itself could not be considered final evidence of parasitism, as the reproductive body might already have been dead at the time it was invaded. For abortive development of sexual apparatus occurs only too frequently in cultures of *Pythium*, often being traceable to such nutritional deficiencies as come into play when, for example, *P. arrhenomanes* Drechsl. (18), *P. periplocum* Drechsl., or *P. sclerotcichum* Drechsl. (10) are grown on unmodified maize-meal agar or on potato agar; the contents of the degenerating oogonia and oospores then being frequently appropriated by such purely saprophytic fungi as may chance to be on hand.

That the moniliaceous form is indeed a parasite became increasingly evident from its constant recurrence in isolation cultures containing oospores or conidia of various species of *Pythium*; from which bodies it manifestly always derived its nourishment. Moreover, it regularly made its appearance in immediate proximity to the pieces of diseased material originally planted on the medium. With the slow extension of its mycelium throughout cultures containing abundant sexual apparatus wholly normal in structural detail, the widening tracts of occupied substratum showed always a steadily increasing proportion of degenerating oospores. This strongly suggested that the hyphomycetous fungus did not wait for the oospores to degenerate spontaneously, but invaded them in their healthy state and actively brought about their destruction.

Closer examination bore out this suggestion. Individual oospores could be found whose central reserve globule, parietal protoplasmic layer and globose or ellipsoidal refringent body still attested a thoroughly normal condition of the protoplast, even after an invading branch had narrowly perforated the enveloping oogonial membrane, had traversed the intervening space, and was perforating the oospore wall itself (Fig. 1, A). From the absence of protoplasmic degeneration previous to invasion, it may be presumed no readily diffusible toxic substance was operative that could be held at all comparable in action to the lethal principle investigated by Weindling (38) and by Weindling and Emerson (39) as a very effective agent in the parasitism of *Trichoderma lignorum* (Tode) Harz on *Rhizoctonia solani* Kühn. Once the invading branch has passed through the oospore wall and into the protoplasmic interior, pronounced changes in internal structure promptly took place. The rather uniformly granular parietal layer and the central reserve globule of homogeneous consistency were both replaced by an irregular arrangement of largish block-like lumps, each of the lumps containing numerous fatty globules of variable sizes. Within this confusion of lumps and granules the progress of invasion by the parasite was always badly obscured. For a period apparently of some duration, it was most difficult to distinguish the haustorial elements from the degenerating protoplasmic materials surrounding them (Fig. 1, H). Following depletion, in large part, of the host protoplasm, the haustorial apparatus could be plainly discerned as a somewhat massive structure composed usually of several swollen lobate elements extending well throughout the cavity of the oospore (Fig. 1, B–G). Once assimilation of the materials within the cavity had been completed, the protoplasmic contents of the haustorial system were withdrawn into the parent hypha, leaving only the empty branching envelope in the collapsing membranous remains of the evacuated sexual apparatus.

Chiefly because the widely distributed damping-off fungus, *Pythium ultimum* Trow, has been obtained more frequently from specimens of diseased plants than any other pathogenic phycomycete, I have observed the moniliaceous fungus most often parasitizing that species in isolation cultures. As might be expected the conidia usually formed in quantity by *P. ultimum* are

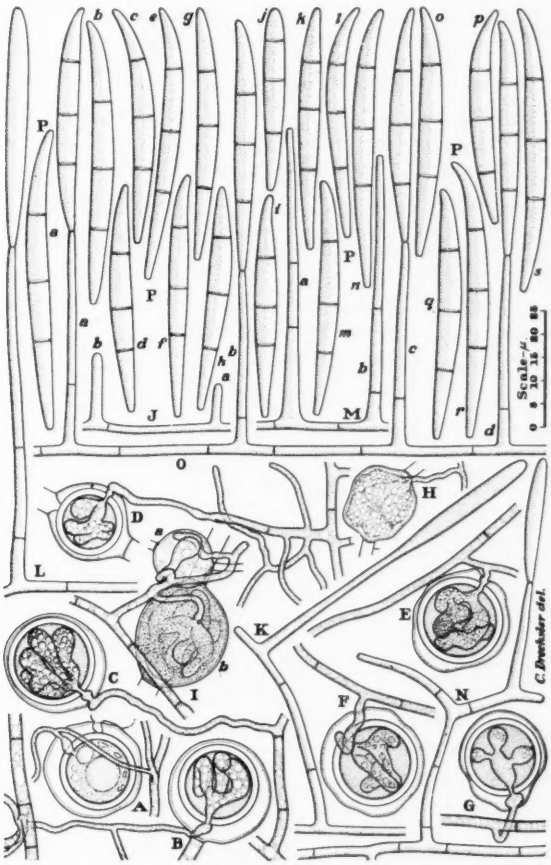

FIG. 1. *Dactylella spermatophaga*; drawn with the aid of a camera lucida to a uniform magnification, from material developed in an isolation culture permeated with *Pythium ultimum*; × 620 throughout. A. Early stage in the invasion of a mature oospore of *P. ultimum* by a hyphal branch of the parasite; the branch having perforated the oogonial wall is penetrating the oospore wall, within which the contents show normal structure—the single large central reserve globule being surrounded by a parietal granular layer that has the single globose refringent body imbedded in it. B–G. Oospores of *P. ultimum* at later stages of invasion by the parasite; as the protoplasmic contents have largely been appropriated, the haustoria are clearly visible as branched lobate structures. H. Conidium of *P. ultimum* soon after invasion by the parasite; the haustorium being concealed in disorganized granular material. I, *a*, *b*. Two invaded conidia of *P. ultimum* from which the contents have been largely taken by haustorial elements of the parasite. J. Two young erect conidiophores, *a* and *b*, arising close together from the same mycelial filament. K. A mature conidiophore, which after producing and shedding one conidium, has given rise below its tip to a lateral branch whereon a second conidium is developing. L. Simple and relatively tall conidiophore bearing a young aseptate conidium. M. Two denuded simple conidiophores arising close together from the same superficial hypha. N. A branched conidiophore giving rise to its third conidium. O. A superficial hypha with four erect simple conidiophores, *a–d*, on each of which a mature conidium is borne. P, *a–s*. Mature conidia showing variations in size and shape.

destroyed much in the same manner as are the oospores, and, perhaps, in view of their less substantial walls, with greater readiness (Fig. 1, H; I, *a*, *b*). The parasite has also been observed operative in hundreds of isolation cultures of *P. debaryanum* Hesse, *P. irregulare* Buisman, and *P. mammillatum*

Meurs, always destroying oospores and zoosporangia in its gradual extension through the substratum. Often, too, it has been observed similarly destructive in isolation cultures of *P. butleri* Subr., *P. vexans* de Bary (=*P. complectens* Braun), *P. complens* Fischer, and *P. arrhenomanes;* as well as in isolation cultures of the species I have described elsewhere (8) under the binomials *P. salpingophorum*, *P. paroecandrum* and *P. oligandrum*. On the other hand, it has never appeared spontaneously in cultures of pythiaceous fungi started from pieces of diseased tissue excised from the interior of such massive plant structures as fruits of the watermelon, *Citrullus vulgaris* Schrad., or as the edible roots of the sweet potato, *Ipomoea batatas* Poir.

After the parasite has been nourished for some time through the destruction of reproductive bodies in isolation cultures of its phycomycetous hosts, it gives rise to scattered conidiophores and conidia. The conidiophores arise as simple erect hyphae (Fig. 1, J, *a*, *b*) that usually complete their development (Fig. 1, L; M, *a*, *b*; O, *a–d*) in producing a single elongate-fusoid triseptate conidium (Fig. 1, P, *a–s*), sometimes straight but more often noticeably curved at the tip like the conidia of various species of *Fusarium*. Some conidiophores put forth a branch to produce individually a second conidium (Fig. 1, K); and, occasionally, the first branch gives rise to a second that then forms a third conidium at its tip (Fig. 1, N). Aside from its less abundant conidial septation, the fungus thus shows, at least in mixed bacterium-laden culture, a marked resemblance to *Dactylella passalopaga* Drechsl., a *Fusarium*-like hyphomycetous form capturing and consuming certain species of testaceous rhizopods (17).

The moniliaceous parasite was isolated by removing its conidia from the supporting hyphae directly to tubes of maize-meal agar; the removal being accomplished by means of minute slices of sterile agar held on a flamed platinum spatula, care being taken to avoid contact with the substratum. Growth on maize-meal agar, as also on other artificial media is relatively slow, radial extension usually not exceeding 1 mm. in 24 hours. The vegetative mycelium, which is almost wholly submerged in the substratum, consists of rather delicate, freely branching hyphae in moderately crowded arrangement. When examined with the naked eye it presents for the most part an irregularly cumulous appearance, except in the central portion, where a more uniformly dense texture is usual (Fig. 2, A–D). About a week after a culture has been started, the central portion begins to show a meager greyish or whitish aerial efflorescence that in the course of time is sparingly extended toward the periphery.

Under microscopic inspection this efflorescence is seen to be composed wholly of asexual reproductive apparatus. Successive production of conidia on individual conidiophores following repeated elongation of the fertile axis, encountered only now and then in bacterium-laden isolation cultures, here comes to elaborate expression. In cultures 30 days old, longish sporophoric hyphae may be found weighted down into somewhat procumbent postures by the conidia, often 10 to 20 in number, that are borne on them mostly at

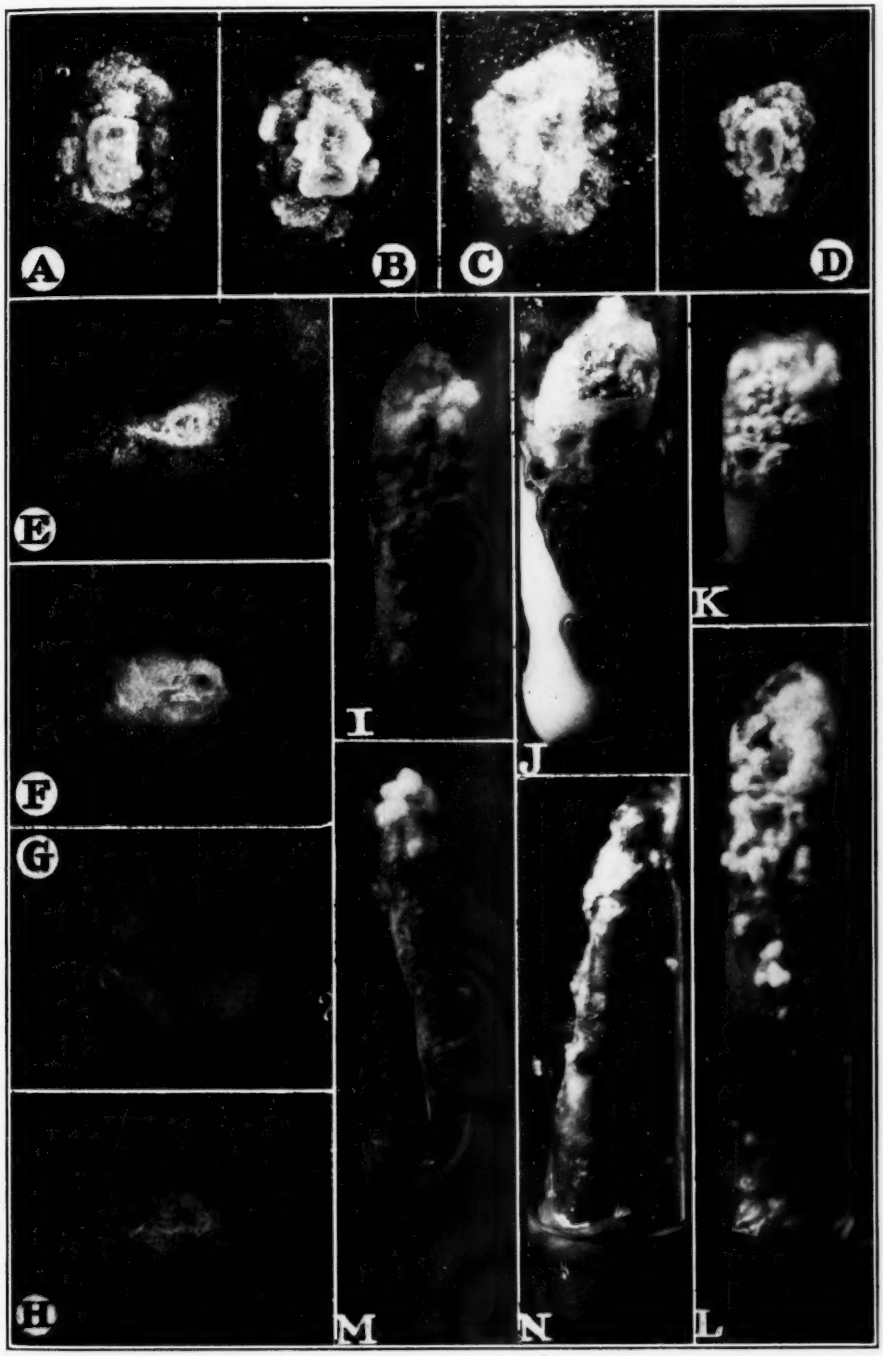

FIG. 2. *Dactylella spermatophaga* photographed from cultures after growing for 13 days at temperatures fluctuating between 25° and 33° C.; all approximately natural size. A–D. Mycelia from maizemeal agar plate cultures, showing rather compact, cumulous texture. E–H. More diffuse mycelia of *D. spermatophaga* resulting from parasitic development in dual culture with *Pythium oedochilum*. I–K. Growth on steamed potato plugs. L–N. Growth on sterilized string-bean pods.

perceptible geniculations and in loose, irregularly spicate arrangement. Sometimes, as often when developing at temperatures between 20° and 25° C., the conidia are conspicuously abnormal in being of small size and irregular shape. Cultures kept at temperatures near 30° C. more often yield conidia similar in shape, size, and septation to those produced in isolation cultures, where, it may be presumed, conditions correspond approximately to those prevailing in nature.

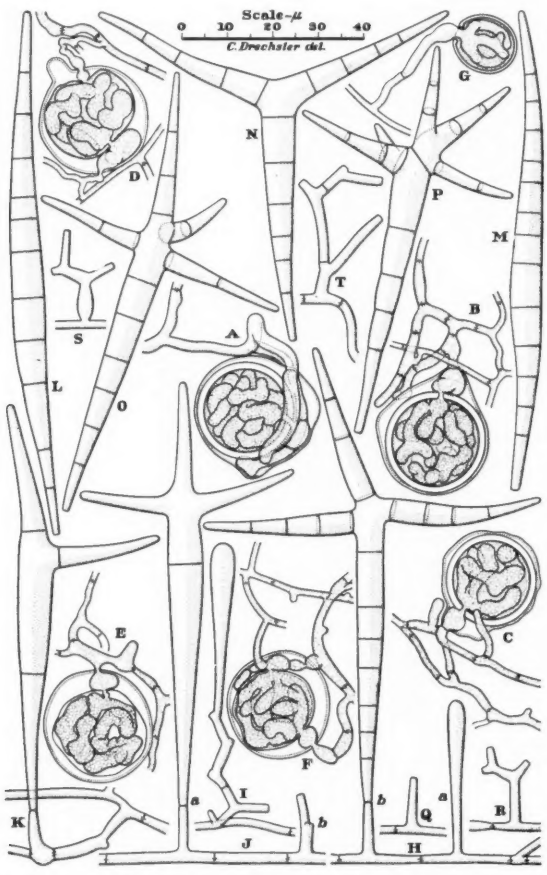

FIG. 3. *Trinacrium subtile*; drawn with the aid of a camera lucida to a uniform magnification, from material developed in an isolation culture permeated by *Pythium butleri;* ×620 throughout. A–F. Oospores of *P. butleri*, each occupied by one or two haustorial systems of *T. subtile*. G. A specimen of the shelled rhizopod *Gcococcus vulgaris* invaded and parasitized by a hyphal branch of *T. subtile*. H. A portion of superficial filament bearing a young conidiophorous process, *a*, and also a conidiophore supporting a mature cruciform conidium, *b*. I. A well-developed conidiophore that after producing and shedding 4 conidia in succession, is giving rise to a fifth. J. Portion of superficial hypha with a conidiophore, *a*, bearing its first conidium, which is of cruciform design and immature, as is evident from the single septum; and close by, a second and denuded conidiophore, *b*, that has produced and shed two conidia. K. Portion of mycelium with a conidiophore bearing a somewhat immature conidium. L–P. Conidia, showing variations in dimensions, shape and septation. Q. A denuded simple conidiophore on which one conidium has been produced. R–T. Branched conidiophores whereon 3, 3, and 4 conidia respectively, have been produced.

On steamed potato plugs (Fig. 2, I–K) and on steamed pods of string bean (Fig. 2, L–N) the parasite likewise shows a slow rate of mycelial extension, but develops in more massive quantity. After 20 days the potato plugs reveal usually an irregularly sculptured, somewhat lichenoid growth, which is dull slaty grey where exposed. For the most part, however, it is clothed either sparingly with a sparse downy efflorescence, or slightly more profusely with a whitish velvety covering. On sterilized string-bean pods the aerial growth becomes aggregated here and there in somewhat raised, lichenoid or cartilaginous, waxy or pale flesh-color masses that, like the subjacent growth, may remain partly bare and partly become overspread with effuse or tufted, whitish or dirty greyish, aerial mycelium. In tubes with the bean pod partly immersed in water, a rather substantial cartilaginous, waxy or flesh-colored shelf may develop at the surface of the liquid. When planted in tubes of steamed rice, the fungus remains for some time scarcely perceptible, but, after 3 or 4 weeks, its development is clearly evidenced by a pale creamy coloration, by a matting together of the substratum, as well as by the presence of whitish downy aerial mycelium on the surface. Bright colorations, of the sort resulting from the growth of many of the familiar species of *Fusarium* associated with plant diseases, have never been observed in cultures of the parasite either on steamed rice or on the two other sterilized vegetable substrata. Neither conidia nor any other kind of reproductive bodies were ever produced in cultures employing one or another of the 3 vegetable materials.

OCCURRENCE OF TRINACRIUM SUBTILE RIESS AS A PARASITE ON OOSPORES

A mucedinaceous fungus, differing rather conspicuously from the one so far discussed, appeared in several maize-meal-agar plate cultures started with pieces of decaying tissue excised from the crowns of some potted spinach plants, *Spinacea oleracea* L., at Arlington Experiment Farm, Rosslyn, Virginia. After a protracted period of hot weather in June, 1936, these plants had become wilted one by one, the wilting in each instance having been followed soon by general collapse of the aerial parts, and death. From nearly all of the excised pieces of tissue, mycelium promptly grew out that in due course gave rise to lobulate zoosporangia and sex organs readily recognizable as those of *Pythium butleri*. To this fungus, often actively destructive in our middle latitudes during spells of high summer temperature, the spinach crown rot is manifestly to be attributed.

When the isolation cultures had become 10 days old, 5 among them showed in the agar adjacent to the tissue fragments numerous oospores of *Pythium butleri* being destroyed by a fungus that penetrated the oogonial and oospore walls and developed a swollen lobulate haustorial system within (Fig. 3, A–F), very much after the manner of the form with triseptate *Fusarium*-like conidia. A difference, apparently of somewhat incidental character, came to light in that the invading branch, after perforating the oogonial envelope, often became markedly inflated in the oogonial cavity, before

proceeding with the perforation of the oospore wall. From the original source in each of the 5 plate cultures the fungus continued to spread steadily until 15 days later it was present throughout the medium in the 100-millimeter Petri dishes, and had destroyed the innumerable oospores of *P. butleri* encountered in the course of its extension. In addition a few specimens of the ovoid testaceous rhizopod *Geococcus vulgaris* Francé were found parasitized by the fungus; a lateral hyphal branch evidently having grown through the mouth of each animal and then given rise inside to a number of swollen assimilative branches (Fig. 3, G).

Sporulation of the parasite took place in moderate quantity here and there over the surfaces of the 5 cultures. Though some of the conidia were elongate-fusoid in shape, they clearly exceeded those of the *Fusarium*-like form in dimensions, sometimes attaining as they did, a length of 115 μ and a diameter of 7.5 μ (Fig. 3, L). Instead of regularly showing 3 septa, these larger uniaxial conidia revealed as many as 13 (Fig. 3, M) or even 15 septa. By far the greater number of spores, however, did not consist of uniaxial bodies, but were branched in various ways. A very considerable proportion were bifurcate,—the proximal element, 4- to 8-septate and often between 40 and 60 μ long, terminating in 2 widely divergent elements, mostly up to 40 μ long and containing about 4 septa, to form a rather symmetrically triradiate structure (Fig. 3, N). An almost equally large number were of cruciform design,—bearing generally somewhat closer to the tip than to the base 2 lateral branches nearly at right angles with the main axis (Fig. 3, H, *b*; J, *a*). A smaller number terminated in 4 (Fig. 3, O) or even in 5 (Fig. 3, P) distal elements.

Conidial production was found to begin with the elongation of a fertile erect aerial outgrowth arising from a superficial mycelial filament (Fig. 3, H, *a*). When this outgrowth had attained approximately its definitive size and its definitive condition with respect to presence or absence of branches, a septum was inserted usually between 5 and 20 μ, often about 10 μ, from the base, thereby setting off stalk from young conidium (Fig. 3, J, *a*; K). Maturation of the delimited spores was accompanied by the insertion of usually 10 to 19 partitions, the number varying approximately with the combined length of the main axis and its branches. After a conidium was formed (Fig. 3, Q) the supporting stalk frequently grew out laterally to produce a second on a fertile extension from 5 to 20 μ in length (Fig. 3, J, *b*); repetition of the process yielding simple or variously branched conidiophores, from the successive geniculations of which the development of 3 (Fig. 3, R, S), 4 (Fig. 3, T), or 5 (Fig. 3, I) conidia could be inferred.

With respect to the morphology of its conidial apparatus the fungus agrees rather well with the description and figures of *Trinacrium subtile* published by Fresenius (23) in 1852. While the nearly symmetrically triradiate conidia (23, Pl. 5, Figs. 14, 15) of the design represented in figure 3, N, were apparently held typical of the species by the early mycologist, he included among his illustrations also a figure of a spore with 4 distal elements

(23, Pl. 5, Fig. 16). The limbs of the triradiate conidia were described as containing 3 to 6 septa and as measuring usually about 40 μ in length, though instances of the proximal limb measuring the equivalent of about 72 μ in length were acknowledged. It is perhaps significant that the species was first recorded as overgrowing the acervuli of another fungus, *Stilbospora* sp., and that the relevant figure (23, Pl. 5, Fig. 17) would seem strongly indicative of a parasitic relationship to the spores of the melanconiaceous form.

The literature bearing on *Trinacrium subtile* is relatively meager. Nearly 3 decades after its original description by Riess and Fresenius, Saccardo published additional figures of the species, wherein conidia of the same triradiate design held typical by Riess and Fresenius were shown both detached and in process of development on conidiophores arising directly from an insect egg (35, Tab. 966). Somewhat later, in a brief diagnosis (36) based more especially on his own material, he characterized the spore elements as being 4- to 5-septate, 25 to 30 μ long and 3.5 to 4 μ wide, and the conidiophores as filiform, 20 μ long and 2 μ wide. Oudemans observed *T. subtile* in a vertical strip of *Pleurococcus* on a beech trunk, *Fagus sylvatica* L., in the Netherlands, without being able to make out any evidence of parasitism. He described the conidia as triradiate; the constituent awl-like radial elements, 25 to 40 μ long, being stated in one account (31) to be divided by 3 or 4 septa into 4 or 5 compartments, and in another account (32) to be divided by 2 to 8 cross-walls into 3 to 9 cells. After Wildeman (40) had listed the fungus in a flora of Belgium, Magnus (30) recorded it on decaying *Berberis* twigs within the region embracing Tirol, Vorarlberg, and Liechtenstein; and more recently Rostrup (34) noted its occurrence on dead branches of *Betula* in Denmark.

The rather small measurements given by Saccardo for width of the radial elements in the conidium of *Trinacrium subtile*, suggests that possibly the Italian fungus recorded on insect eggs may have represented a species less robust than the one found destructive to the oospores of *Pythium butleri*. In any case, however, the oospore-destroying parasite conforms closely to the description and figures published by Fresenius; and its conidial septation shows a range of variation in fair consonance with Oudemans' somewhat variant statements regarding this feature. It is accordingly referred, at least provisionally, to the species originally discovered by Riess and adequately described by him in text quoted by Fresenius.

TAXONOMIC POSITION OF TRINACRIUM SUBTILE AND DACTYLELLA SPERMATOPHAGA

The very evident parallelism in parasitic development between the two Hyphomycetes destructive to oospores leaves scarcely any doubt that they are closely related to one another. A presumption of intimate kinship is supported by the resemblance that the uniaxial conidia intermingled with the branching spores of *Trinacrium subtile* bear to the smaller triseptate conidia of the other species, and also by the similarity of the two species with

respect to the production of successive conidia following repeated elongation of the conidiophore. Now, in morphology of conidial apparatus the fungus with triseptate spores shows unmistakable parallelism and evident kinship especially with *Dactylella passalopaga*. Since *D. passalopaga*, as was pointed out earlier, manifestly belongs to an interrelated series of Hyphomycetes including in its known membership various species of *Trichothecium*, *Arthrobotrys*, *Dactylella*, *Dactylaria*, *Triposporina*, *Pedilospora*, and *Tridentaria* predacious either on nematodes or on rhizopods, it follows that the 2 oospore-destroying forms must also belong in the same natural alliance, despite the generally different biotic relationship pertaining to both alike. For that matter, the divergence in biotic relationships is not so pronounced as to imply mutual exclusiveness in these relationships; reciprocal overlapping coming to light through the occasional parasitism on the one hand, of *T. subtile* on *Geococcus vulgaris*, and through occasional destruction (20, p. 510), on the other hand, of *Pythium* oospores by the nematode-capturing *Dactylella gephyropaga* Drechsl. Pertinent significance, moreover, may well attach to the fact that all known congeners of *Pedilospora dactylopaga*, a form I described (9) as predacious on the shelled rhizopods *Difflugia globulosa* Duj. and *Trinema enchelys* Ehrenb., were reported on other fungi—*P. parasitans* Höhn. (25) being recorded on *Helotium citrinum* (Hedw.) Fr., *P. ramularioides* Bubák (4) on *Bispora pusilla* Sacc., *P. episphaeria* Höhn. (26) on *Nectria cucurbitula* Fr., and *P. zaczewskii* Gizhits'ka (24) on *Acrothecium tenebrosum* Preuss.

Considered from a morphological viewpoint, the 2 oospore-destroying fungi fit acceptably in the predacious series. The short conidiophores of *Trinacrium subtile* invite comparison with the similarly stubby fertile hyphae of the amoeba-capturing *Dactylella tylopaga* Drechsl. (14); its occasional uniaxial conidia suggest the elongated many-septate spores of *D. leptospora* Drechsl. (20); and its more numerous variously branched conidia recall the homologous branched structures of *Pedilospora dactylopaga*, *Triposporina aphanopaga* Drechsl. (20), and *Tridentaria carnivora* Drechsl. (21). Repeated prolongation of conidiophores with the production of additional conidia, prevails in conspicuous measure among some of the capitate nematode-capturing forms, as notably in *Arthrobotrys superba* Corda, *A. oligospora* Fres., *A. conoides* Drechsl. (20), and *Dactylaria polycephala* Drechsl. (20); yet certainly, under especially favorable conditions, is not wholly absent among the predacious species of *Dactylella*, even though this genus is defined—and with respect to development on natural substrata, properly defined—as producing solitary acrogenous conidia. Indeed, such repeated prolongation is found not altogether rarely, even in bacterium-laden cultures of *D. passalopaga*, with which fungus, as has been stated, the smaller oospore-destroying parasite would seem most intimately allied. This parasite, having apparently not been described hitherto, possibly because its commonplace appearance and its very meager development of reproductive apparatus invite little attention, is accordingly presented as a new species of *Dacty-*

lella; a term meaning "eating seeds" being deemed a suitably descriptive specific name.

Dactylella spermatophaga sp. nov.

Sparsa; mycelium ramosum, hyphis sterilibus hyalinis, .8–4 μ crassis, aliis angustis, flexuosis, filiformibus, parce septatis, aliis latioribus, torulosis, crebro septatis,—quibusvis ramulum tenuem in corpora perdurantia Pythiacearum saepe intrudentibus, haustorium obesum ramosum plerumque plus minusve lobosum intus evolventibus quod protoplasma assumit; hyphis fertilibus hyalinis, erectis, septatis, simplicibus vel interdum parce ramosis, vulgo 35–75 μ altis, basi 2.5–4 μ crassis, sursum leniter attenuatis, apice 1–2 μ crassis, ibi unicum conidium ferentibus, atqui subinde identidem repullulantibus, 10–15 additicia conidia deinceps gerentibus, tum usque 300 vel 500 μ longis et fere aliquid procumbentibus. Conidia hyalina, elongato-fusoidea, recta vel saepe praecipue in parte superiore curvata, basi rotunde truncata, apice rotundata, 35–65 μ (saepius circa 50 μ) longa, 3.8–5.2 μ (saepius circa 4.5 μ) crassa, vulgo triseptata, rarius biseptata vel quadriseptata.

Hyphas, conidia, zoosporangia, oogonia, praecipue oosporas multarum specierum Pythiacearum (magnam partem Pythii et Phytophthorae) interimens, habitat late in terra et in materiis plantarum putrescentibus, prope Beltsville, Maryland et in Arlington, Virginia.

Sparse; mycelium abundantly branched; vegetative hyphae hyaline, .8 to 4 μ wide, some narrow, flexuous, filiform and sparingly septate, others wider, toruloid and more closely septate,—whether of one type or the other, often thrusting a narrow branch into the resting reproductive bodies of Pythiaceae, and then, in each instance, giving rise inside to a rather bulky haustorium consisting of more or less lobulate branches usually 2 to 5 μ wide, yet sometimes as much as 6 or 7 μ in width; conidiophores, hyaline, septate, simple or sometimes somewhat branched, commonly 35 to 75 μ high, 2.5 to 4 μ wide at the base, tapering upward to a width of 1 to 2 μ at the tip, whereon is borne a single conidium, —but when developing under especially favorable conditions, often on repeated elongation giving rise at successive intervals to 10 or 15 additional conidia, thereby attaining lengths of 300 to 500 μ and becoming more or less procumbent. Conidia hyaline, elongate spindle-shaped, roundly truncate at the base, narrowly rounded at the tip, straight, or more especially in the distal portion, curved, 35 to 65 μ (average about 50 μ) long, 3.8 to 5.2 μ (average about 4.5 μ) wide, commonly containing 3, more rarely 2 or 4 cross walls.

Parasitizing hyphae, conidia, zoosporangia, oogonia and more particularly oospores of many species of Pythiaceae (mostly species of *Pythium* and *Phytophthora*), it occurs widely in the soil and in decaying plant remains near Beltsville, Md., and in Arlington, Va.

It must be admitted that with respect to reproductive habit neither *Dactylella spermatophaga*, nor certainly *Trinacrium subtile*, shows much direct resemblance to *Arthrobotrys oligospora* Fres., the species most familiarly exemplifying the predacious hyphomycetes; the connection being traceable only rather indirectly through a number of forms embodying transitional types of conidial apparatus. That the chain of similarities here concerned, though perhaps appearing at first somewhat far-fetched, is not beyond the scope of a well-integrated group, becomes manifest from its parallelism with an analogous train of resemblances extending more particularly through the noncatenulate members of the Zoopagaceae, an unquestionably natural family of conidial phycomycetes destructive to nematodes and rhizopods. Thus, the branched conidium produced on the short conidiophore of *Trinacrium subtile*, shows an engaging analogy to the appendaged and virtually sessile conidium of *Acaulopage tetraceros* Drechsl. (12) or of *A. acanthospora* Drechsl. (22); the elongated conidium and moderately developed conidiophore of *Dactylella spermatophaga* recalls the similarly proportioned homologous structures of *Stylopage rhabdospora* Drechsl. (16); the broadly ellipsoidal or obovoid conidia and tall conidiophores of *Dactylella bembicodes* Drechsl. (20), *Dactylella gephyropaga*, and *Trichothecium polybrochum* Drechsl. (20) are

reminiscent of the conidial apparatus in *S. hadra* Drechsl. (13) and *S. leiohypha* Drechsl. (15); while to extend the correspondence, the monocephalous capitate moniliaceous forms as, for example, *Dactylaria candida* (Nees) Sacc. and *Arthrobotrys dactyloides* Drechsl. (20), show a striking similarity of habit to *S. cephalote* Drechsl. (22).

An alternative disposition of the two oospore-destroying forms is, of course, suggested by the remarkable structural resemblance that the conidia of *Dactylella spermatophaga* bear to the conidia of some species of *Fusarium*—a resemblance that gains in possible import from the known occurrence of many species of *Fusarium* on other fungi (41, pp. 302–305). However, assimilation of *D. spermatophaga* to *Fusarium* encounters a serious obstacle in the failure of the fungus to produce conidia otherwise than terminally on discrete aerial hyphae. Clements and Shear (6, p. 402), it is true, have ventured to subsume under *Fusarium* the genus *Rachisia* represented by a single species, *R. spiralis* Lindner, which, after developing endoparasitically in vinegar eels (*Anguillula aceti* Ehrenb.), produces falcate conidia laterally on external discrete rachiform filaments. Unfortunately, Lindner (28) omitted to state whether cross walls are present or absent in the hyphae and conidia of *R. spiralis*; yet, the rachiform conidiophores pictured by him reveal a most persuasive similarity to those of *Graminella bulbosa* recently described by Léger and Gauthier (27) from the rectal cuticle of *Bactis* larvae. Indeed, the resemblance is so striking that, until further information is obtained, Lindner's remarkable parasite might well be extricated from any association with *Fusarium* and placed, even if only somewhat tentatively, in the Harpellaceae, a singular family of conidial phycomycetes occurring especially on the peritrophic membrane and rectal cuticle of the aquatic larvae of various insects.

DACTYLELLA SPERMATOPHAGA IN DUAL CULTURE WITH VARIOUS OOMYCETES

After *Dactylella spermatophaga* had been obtained in pure culture, the scope of its parasitism was further explored by growing it in dual culture together more especially with different pathogenic oomycetes that had not previously been observed being attacked in isolation cultures. In order that spontaneous degeneration of the host fungi may not be held to have impaired the trustworthiness of observations recorded here, only results will be considered that could be obtained by the use of maize-meal agar media clear enough to permit satisfactory inspection throughout and thereby to make possible the confirmation of normal development in the particular oomycetes under trial. Among the species of *Phytophthora* thus tried out, oospores of undoubtedly normal structure were produced in quantity by *P. cactorum* (Leb. & Cohn) Schroet. and *P. megasperma* Drechsl. After their maturation the oospores of both species were successfully parasitized by *P. spermatophaga*, and that despite the thickness of their protective walls. The clustered globose chlamydospores of *P. cinnamomi* Rands were likewise abundantly

attacked and destroyed, as were also the more solitary globose thick-walled chlamydospores or "resting zoosporangia" of *P. parasitica* Dast.

In dual culture with *Dactylella spermatophaga*, oospores of *Aphanomyces cuteiches* Drechsl., a pathogen often responsible for serious root rot of peas (*Pisum sativum* L.) during wet seasons, were freely invaded and exhausted of contents. Neither the thick oogonial membrane characteristic of this water mold, nor the substantial oospore wall, appeared to offer any effective barrier to entrance by the hyphomycetous parasite. Rather often, to be sure, the oospore was reached not by perforation of the oogonial envelope, but by penetration of the antheridial wall, followed by elongation of the infective hypha through the antheridial chamber and down the fertilization tube. This easier mode of invasion is apparently facilitated by the frequent presence of 3, 4, or 5 male organs about an oogonium, by the fairly large size of these organs, and by their retention, sometimes, of granular protoplasm, even at relatively late stages of development.

When *Pythium graminicolum* Subr., *P. dissotocum* Drechsl., and *P. periilum* Drechsl., each isolated (8, 18) from diseased roots of sugar-cane, *Saccharum officinarum* L., were grown in dual culture with *Dactylella spermatophaga*, the numerous oospores of correct internal structure produced by them were parasitized in large numbers. Such parasitism was evident also in dual cultures of *D. spermatophaga* and *P. myriotylum* Drechsl., a species occurring widely in our southeastern States as the cause of a blossom-end decay of watermelon fruits (8), as well as of a cottony rot of the fruits of cucumber, *Cucumis sativus* L., closely resembling "cottony leak" caused by *P. butleri*. With no less success *D. spermatophaga* likewise parasitized the oospores of *P. acanthicum* Drechsl. and *P. periplocum*, the 2 forms with characteristically spiny oogonia that are most frequently found associated with blossom-end rot of watermelon fruits in Maryland, Indiana, and Missouri (8). In dual culture on slightly acidulated maize-meal agar containing considerable maize-meal sediment in suspension, the hyphomycetous parasite abundantly destroyed the oospores of *P. scleroteichum*, one of the species frequently causing in edible sweet-potato roots the marbled decay known as "mottle necrosis."

Among morphological modifications to be observed in the sexual apparatus of oomycetes, few are more formidable in aspect or present more of an appearance suggesting a protective function than the sturdy, often characteristically mammiform protuberance normally besetting the oogonia (8) of *Pythium polymastum* Drechsl. and *P. mastophorum* Drechsl. Yet, *Dactylella spermatophaga*, in mixed culture with these impressive and rather rare representatives of the genus *Pythium*, thrusts its infective branches through the sturdily mamelonated oogonial envelopes without apparent difficulty, to reach the smooth, globose oospores within; the infective branches then, of course, individually perforating the oospore wall and extending a lobulate haustorium through the protoplasmic interior. Equally ineffective for protection against invasion by the hyphomycetous parasite is the prominently

echinate oogonial wall of *P. anandrum* Drechsl., a species remarkable alike for its consistent parthenogenesis and for the *Phytophthora*-like shape of its repeatedly proliferous sporangia (8). The handsome parthenospores of the species are readily invaded and destroyed on their development in dual culture with *D. spermatophaga*.

Further testimony to the efficiency of *Dactylella spermatophaga* in penetrating sturdy host structures is furnished by its more than ordinarily profuse development when grown in dual culture with any one of the 4 closely interrelated forms I described (8) under the names *Pythium helicoides*, *P. oedochilum*, *P. polytylum*, and *P. palingenes*. In their asexual reproduction these forms follow the development early set forth by de Bary (1) in the description of his *P. proliferum*, giving rise, as they do on appropriate irrigation, to repeatedly proliferous terminal sporangia that individually discharge their undifferentiated contents through an evacuation tube into a vesicle, there to be fashioned into zoospores (Fig. 4, A, B). Much greater distinctiveness attaches to their sexual apparatus, not only in that the elongated antheridia are applied lengthwise to the oogonium rather than apically, as in most species of *Pythium*, but, also, in that the mature oospore regularly reveals imbedded in its somewhat turbidly granular protoplasmic contents usually about a score of reserve globules and about a half dozen refringent bodies (Fig. 4, F, G), rather than only the single reserve globule and single refringent body more generally present elsewhere among the monospermous oomycetes of the genera *Pythium*, *Phytophthora*, *Aphanomyces*, and *Plectospira*. Of particular relevance to the present discussion is the fact that the oospore wall in each of the 4 species usually averages about 2.5 μ or more in thickness, besides presenting an appearance of marked induration through its frequently yellow or yellowish coloration. Yet, after penetration of the oogonial envelope, this conspicuously substantial wall is perforated without apparent difficulty by the invading branch of *D. spermatophaga*; extension of a massive lobulate haustorial system throughout the protoplasmic interior then ensuing (Fig. 4, H and K), as in the less stoutly incased oospores of the species more frequently isolated.

As *Pythium oedochilum*, when grown on maize-meal agar, is very dependable in producing—and rather abundantly, too—sexual apparatus of uniformly normal structure, it makes an especially satisfactory host in dual culture with the hyphomycetous parasite. On encountering a mycelium of *Dactylella spermatophaga*, many of its vegetative hyphae are invaded over stretches of variable lengths and locally exhausted of their contents (Fig. 4, C–E). With the appearance later of zoosporangia, these are parasitized (Fig. 5, A) no less successfully than are the hyphae and oospores. Thus richly nourished, the parasite gives rise to its most luxuriant display of simple or somewhat branched conidiophores (Fig. 5, B, C) bearing numerous conidia (Fig. 5, D, *a–n*) comparable in shape and size to those produced in pure culture only under exceptionally favorable conditions. Frequently the conidia anastomose with their supporting hyphae previous to normal dis-

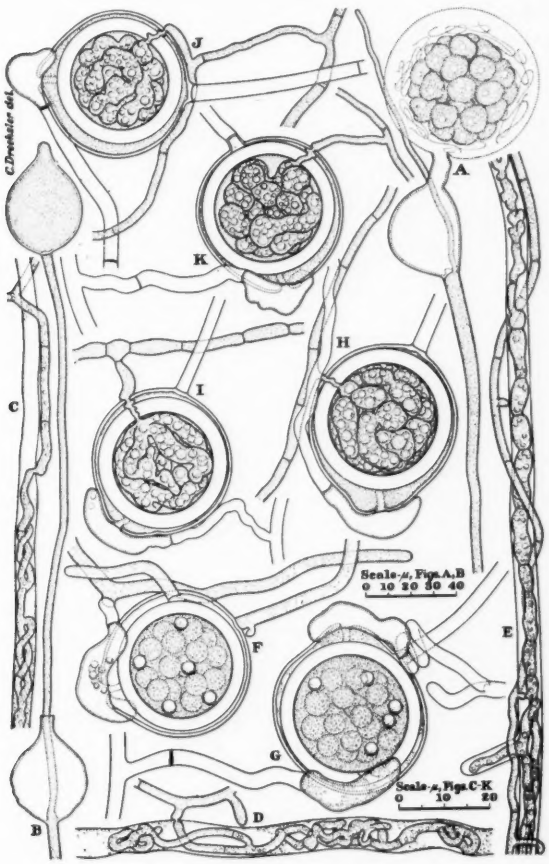

FIG. 4. *Dactylella spermatophaga* and *Pythium oedochilum*; drawn with the aid of a camera lucida, mostly from material developed in dual culture on maizemeal agar. A. A zoosporangium of *P. oedochilum* showing zoospores being formed in a vesicle at the tip of an evacuation tube; the supporting hypha is continuing growth from immediately below the sporangium; ×310. B. Two zoosporangia of *P. oedochilum*, the distal one borne on an axial prolongation of the conidiophore supporting the proximal one; ×310. C–E. Hyphae of *P. oedochilum* parasitized by filaments of *D. spermatophaga;* ×620. F, G. Normal mature sexual apparatus of *P. oedochilum*, showing the internal organization characteristic of the *helicoides* series in *Pythium*—multiple reserve globules and multiple refringent bodies being imbedded in murkily granular protoplasm; spiral involvement of the oogonial stalk by the branch supporting one of the antheridia, in the manner shown in G, occurs infrequently in the species, though regularly present in *P. helicoides;* ×620. H–K. Infected sexual apparatus of *P. oedochilum;* the oospore in each unit having been occupied by a rather massive branching haustorium of *D. spermatophaga*, after successive perforation of the oogonial and oospore walls; ×620.

articulation (Fig. 5, E); or, falling on the substratum, unite vegetatively with one another (Fig. 5, F), sometimes to give rise to secondary conidia (Fig. 5, G). To the naked eye, as might be expected, the parasitic mycelia of *D. spermatophaga* present a more diffuse appearance than mycelia developing in pure culture, rarely showing any suggestion of cumulous texture (Fig. 2, E–H).

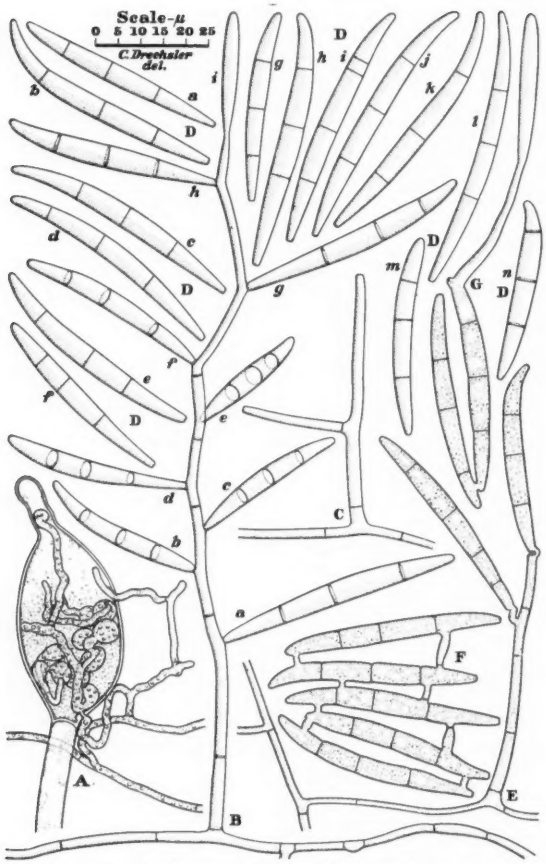

FIG. 5. *Dactylella spermatophaga;* drawn with the aid of a camera lucida to a uniform magnification, from material developed in dual culture with *Pythium oedochilum* on maizemeal agar; ×620 throughout. A. A sporangium of *P. oedochilum* invaded and nearly depleted of protoplasmic contents by the parasite. B. A conidiophore on which have been produced successively 8 conidia, *a–h*; a ninth conidium, *i*, being in course of development at the apex. C. Portion of hypha with a branched conidiophore denuded of its two conidia. D, *a–n*. Conidia, showing variations in size, shape and septation. E. Portion of mycelium with a conidiophore whereon have been produced 2 conidia, both of which have become united vegetatively with the supporting hypha previous to their disarticulation therefrom. F. Five conidia that after falling on the substratum have become united vegetatively through fusion by means of germ tubes. G. Two fallen conidia that have fused vegetatively; from one of them a conidiophore has been produced that is giving rise to a secondary conidium.

THE RÔLE OF DACTYLELLA SPERMATOPHAGA IN THE BIONOMICS OF ROOT-ROTTING OOMYCETES

Though *Dactylella spermatophaga* is unquestionably capable of destroying mycelial filaments of numerous species of *Pythium*, the destruction of vegetative hyphae would seem on the whole far too meager to be of any great importance in the bionomics of the oomycetous hosts. Even the slower-growing species among the phycomycetes subject to attack show rates of mycelial extension from 10 to 15 times faster than that of the parasite, while some hosts, as, for example, *P. debaryanum*, *P. ultimum*, and *P. butleri*,

exceed the linear or radial growth of *D. spermatophaga*, 50 to 75 times. A mycelium extended by a pathogenic oomycete through a sizable portion of root or stem, or through any other rather bulky mass of substratum, would, for the most part, therefore, presumably have ample time to form reproductive bodies before it could be overtaken by the parasite.

Though *Dactylella spermatophaga* may appear rather innocuous in its occasional invasion of vegetative hyphae, as also in its occasional interception of reproductive development, its unceasing destruction of mature oospores and conidia must almost certainly reduce the longevity of its phycomycetous hosts. Evidently widely distributed in the soil, as well as in plant materials decaying moistly in contact with the ground, and freely attacking the most resistant of soil-borne reproductive bodies through which the many root-rotting oomycetes are enabled to persist from season to season, *D. spermatophaga* must be considered an organism with very real potentialities for promoting soil sanitation. Since, in the persistence of oospores, and accordingly also in programs of crop rotation governed thereby, periods of time reckoned in years rather than in days, weeks, or months, are involved, the relatively slow growth of the parasite would seem not seriously incompatible with its efficiency as a hygienic agent. Even in much shorter periods, oospores at first inaccessible to attack because of the massiveness of the phanerogamic structures occupied, become subject to invasion with the disintegration of the substratum. Thus, while the innumerable oospores formed by *Pythium acanthicum* and *P. periplocum* in diseased watermelon fruits may enjoy protection for perhaps a week or two, decomposition of the affected material soon exposes them to the action of the parasite.

OTHER ORGANISMS ANTAGONISTIC TO ROOT-ROTTING OOMYCETES IN ISOLATION CULTURES

Apart from *Trinacrium subtile* and *Dactylella spermatophaga*, oospores of root-rotting phycomycetes in isolation cultures have been found undergoing destruction by amoebae and shelled rhizopods. The various amoebae implicated vary greatly in size. Some are so small that after ingesting a single oospore of *Pythium ultimum* or *P. debaryanum* they appear individually as only a rather thin enveloping layer of protoplasm. Others are large enough to move about briskly after engulfing a dozen oospores of the two damping-off fungi mentioned. Somewhat curiously, the small species of *Amoeba* seem generally much more rapid in killing and digesting oospores devoured by them than are the larger congeneric forms. Oospores have likewise been found ingested by the robust, large-mouthed testaceous rhizopod *Arcella vulgaris* Ehrenb.; or, again, have been observed in process of being exhausted of their degenerating contents by the testaceous rhizopods *Geococcus vulgaris* Francé and *Euglypha denticulata* Brown, following, in each instance, perforation of the oospore wall within an area circumscribed by the mouth of the shelled animal orally applied to it (17, pp. 400–401; 19, p. 244). On the whole, however, the aggregate destruction of oospores seen accom-

plished in isolation cultures by all protozoans together, while sometimes not inconsiderable, seems hardly deserving of comparison with the destruction found achieved by *D. spermatophaga*.

Butler (5, p. 109) directed attention to the somewhat unexpected occurrence of chytridiaceous parasites in soil-inhabiting species of *Pythium*. The pure-culture technique whereby affected portions of root or stem are placed on firm agar culture media, ordinarily gives little encouragement to the development of any organisms able to spread only through locomotion of zoospores. Nevertheless, some slight opportunity for the development of such organisms is often provided by the accumulation of water in a minute pool immediately surrounding the planted decaying material. In the limited area covered by the pool, *Pleolpidium inflatum* Butl., with its large and immensely prolific zoosporangia, can sometimes be found actively parasitizing *Pythium* filaments. Very little free water would seem to suffice for the propagation of *Pleolpidium irregulare* Butl., as this species has been found extending itself successfully in maize-meal agar cultures of *Pythium irregulare* on the surface of which no deposit of liquid was visible. The chytridiaceous parasite was for years maintained in culture free of bacteria and all extraneous organisms by merely transferring it with its fungus host from one tube of firm agar medium to another. It singles out for invasion especially the growing oogonia, which organs are often conveniently identifiable by the characteristic digitations borne on them (5, Pl. 8, Fig. 11); so that its parasitism, if not effecting the destruction of oospores already formed, yet operates, in part, like the parasitism of *Dactylella spermatophaga*, by preventing their formation.

The generally unfavorable effect of bacterial contamination on cultures of most pythiaceous and saprolegniaceous fungi has long been annoyingly familiar both among students of the aquatic microflora and among investigators dealing with plant diseases. Such contamination, being here far more pertinacious than in cultures of most other fungi, usually requires extra effort for its elimination; wherefore, indeed, different special methods of purification have been devised, including some made known in recent years by Brown (3), Volkonsky (37), Machacek (29), Blank and Tiffney (2) and Raper (33). Yet in investigations on root rots and other related plant diseases, discomfiture more serious than any resulting from an unhappy intrusion of bacteria in isolation cultures, has often come in repeated failure to obtain on agar or other artificial medium any growth whatever of the causal parasite concerned; so that in the absence of any isolation culture from which a correct beginning might be made, either no likely pathogenic agent could be uncovered at all, or the disease was (and it may be suspected in many instances still is) wrongly attributed to an adventitious organism. According to the relevant literature, species of *Phytophthora* that in nature develop at relatively high temperatures in the softer succulent tissues of herbaceous or, for that matter, of woody plants, have often been found difficult to get started from pieces of diseased tissue placed on artificial sub-

strata; as have also most disease-producing species of *Aphanomyces* and some root-rotting species of *Pythium* other than the common damping-off forms distinguishable among phycomycetous parasites for their vigorous and extensive extramatrical growth—*P. ultimum, P. debaryanum, P. irregulare, P. mammillatum,* and *P. butleri.*

Microscopic examination of tissues undergoing active invasion by species of oomycetes, not readily started in isolation cultures, has very generally shown the difficulty to lie in a debilitated condition of the parasitic mycelium, evidently attributable, in the main, to the presence of putrefactive bacteria. During warm periods especially, the motile saprogenous bacteria that habitually accompany the phycomycetous filaments in their courses through host tissue, become so active that often many can be seen swarming about the very tips of the invading hyphae, just as under similar conditions they swarm about the tips of superficial hyphae of growing mycelia in isolation cultures. Now, whereas on firm agar media development of bacteria is limited, for some time at least, to the surfaces, so that submerged filaments are protected from the products of putrefaction save as these gradually diffuse through the intervening gel, in invaded plant tissues the multiplying bacteria permeate the substratum throughout, thereby exposing all filaments to direct contact with the saprogenic substances. As a result protoplasmic degeneration becomes evident everywhere except in the youngest hyphae in the forefront of the advancing mycelium. Sometimes, in fact, bacterial development appears to overcome even the filaments in the van of an infection; partly decayed specimens of watermelon fruits, for example, having been found wherein the advance of one or another of the different parasitic *Pythium* species causing blossom-end rot seemed to have been stopped outright. Where remnants of mycelium survive in the marginal portions of a lesion, they nearly always can be induced to grow out conveniently into an agar substratum if first the accumulated products of putrefaction are leached away by bathing the affected tissues in several changes of fresh water, and bacterial development, after the transfer to agar, is discouraged through the removal of all free water previously by pressing the material between pieces of sterilized filter paper (7).

Indeed, within their host plants no less than in artificial culture, the whole behavior of pythiaceous and saprolegniaceous parasites betrays, as a general fundamental character, an incapacity for thriving, or even for long enduring vegetatively in a fouled substratum. With their markedly rapid rate of mycelial extension some species of *Pythium* are enabled to thrust their assimilative hyphae into fresh unfouled masses of plant material, moribund or newly dead, ahead of competing fungi of slower growth, and there to build up a vegetative thallus of sufficient bulk for the development of oospores before the increasing putrefaction brought on by the accompanying bacteria causes protoplasmic degeneration on a serious scale. Despite their sensitiveness to putrefaction such fast-growing species of *Pythium* frequently occur, therefore, in semi-parasitic and saprophytic relationships as well as in truly

parasitic relationships resulting from the invasion of succulent living structures. It may be presumed, on the other hand, that species of *Phytophthora* with their slower mycelial growth are at a great disadvantage in any competitive invasion of plant tissues normally moribund or otherwise deprived of effective resistance; and are consequently, for the most part, limited in their development under natural conditions to tissues newly invaded and freshly killed by them. Their ready saprophytic development in pure culture on a wide variety of artificial and natural substrata, yet always in the absence of putrefactive organisms, must accordingly not be considered inconsistent with their habitual occurrence in nature as parasites. With respect to nutrition, then, the root-rotting oomycetes in general seem comparable to the predacious hyphomycetous fungi and the two related oospore-destroying forms herein discussed; though a notable difference is presented in that, while the hyphomycetous fungi apparently cannot take nourishment from a foul substratum, their mycelia readily endure immersion in putrescent slime. As was pointed out earlier (11) *Pythium anandrum*, *P. mastophorum*, and *P. polymastum* are appreciably less sensitive to bacterial contamination than the main mass of root-rotting oomycetes; their capacity for producing zoospores in water rather generously laden with bacteria, as well as their somewhat aberrant morphology, perhaps being indicative of special ecological relationships. However, the moderate tolerance of these species to bacterial contamination is not to be confused with the thoroughgoing adaptation to foulness shown by members of the truly saprophilous genus *Pythiogeton*, which grow luxuriantly and produce zoospores abundantly in heavily putrescent slimes.

From some writings on damping off the suggestion is conveyed that addition of stable manure is undesirable, not only because infectious material may be introduced and tender growth favorable to infection encouraged thereby, but also because after such addition the seedling parasites might be able to permeate the soil more readily by developing on the bits of dung distributed through it. Misgivings on the latter score would seem excessive, as certainly the behavior of the pythiaceous damping-off organisms in the laboratory is not such as to justify any fear that they might spread anywhere on a substratum as foul as dung. On the contrary, their behavior suggests that if a seedbed could be kept continuously infiltrated throughout with putrescent substances, development of destructive mycelia from resting conidia and oospores might be effectively prevented. Indeed, it appears within the realm of possibility that where soil sterilization by steam or chemicals is inexpedient, some control of damping off might be achieved by watering seedling flats exclusively with a putrescent infusion not objectionably rich in nitrogenous substances, such as could be conveniently prepared by the retting of herbaceous waste materials.

SUMMARY

Of 2 moniliaceous fungi found vigorously parasitic on oospores of pythiaceous root-rotting organisms, one with mostly triradiate and cruciform

conidia is identified as *Trinacrium subtile*; the other with regularly triseptate *Fusarium*-like conidia is described as a new species under the binomial *Dactylella spermatophaga*. Despite conspicuous differences in conidial design, the 2 hyphomycetous parasites appear closely related to one another. Because of its failure to produce compound conidial apparatus, and in view of its resemblance to *D. passalopaga*, a rhizopod-capturing form, as well as to *D. leptospora*, a nematode-capturing species, *D. spermatophaga*, together with *T. subtile*, is considered related taxonomically to the predacious series of hyphomycetes rather than to the genus *Fusarium*.

Dactylella spermatophaga has been observed to appear spontaneously in numerous isolation cultures started from diseased portions of many host plants obtained in a number of different localities representing collectively a wide variety of environmental conditions. In these cultures it was found destroying oospores of *Pythium arrhenomanes*, *P. butleri*, *P. complens*, *P. debaryanum*, *P. irregulare*, *P. mammillatum*, *P. oligandrum*, *P. paroecandrum*, *P. salpingophorum*, *P. ultimum*, and *P. vexans*. Grown in dual culture with other oomycetes it parasitized abundantly also the oospores of *Pythium acanthicum*, *P. anandrum*, *P. dissotocum*, *P. graminicolum*, *P. helicoides*, *P. mastophorum*, *P. myriotylum*, *P. oedochilum*, *P. palingenes*, *P. periilum*, *P. periplocum*, *P. polymastum*, *P. polytylum*, *P. scleroteichum*, *Phytophthora cactorum*, *Phytophthora megasperma*, and *Aphanomyces euteiches*. Infection is accomplished by perforation successively of the oogonial and oospore walls, followed by development within the oospore of a branched, somewhat lobate, and rather massive haustorium that appropriates the protoplasmic contents.

Because of its evident capacity for widespread destruction of oospores, on which bodies root-rotting oomycetes mainly depend for survival from season to season, *Dactylella spermatophaga* presumably serves as an effective agent in promoting soil sanitation over extended periods of time. A similar rôle in the long-time bionomics of soil-borne oomycetes is played by various amoebae and testaceous rhizopods. Parasitic chytrids, on the other hand, operate not so much to destroy oospores already formed, as they operate to prevent their development; while putrefactive bacteria exert a pervasively debilitating effect on the mycelium of root-rotting oomycetes,—an effect entailing, under natural conditions, the virtual restriction of slow-growing oomycetous forms to strictly parasitic relationships, though permitting the occurrence of faster-growing forms in saprophytic and semiparasitic as well as wholly parasitic relationships.

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MINT ANTHRACNOSE

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The disease referred to herein as mint anthracnose was brought to the author's attention in 1931, when specimens of infected black peppermint, *Mentha piperita* L. var. *vulgaris* Sole, were received from 2 sections in northern Indiana. While there is no record of the occurrence of the disease prior to 1931, mint growers had observed it a number of years earlier and called it leopard spot. During the past 3 years mint anthracnose has been found in many fields of peppermint and also in a field of spearmint. The degree of infection and amount of injury to the plants varied from very slight to severe. In nearly all cases the disease was more prevalent in fields of old established peppermint than in newly planted fields. However, in 1933, one-third of the plants in a 20-acre field of first-year peppermint were found infected. Nelson[2] briefly described the disease (leopard spot), which was first observed in Michigan in 1935.

DESCRIPTION OF THE DISEASE

Small brown spots are the first symptoms of the disease on young stems and stolons of black peppermint (Fig. 1, A). Later, these areas enlarge and become somewhat oval with a light, or ash colored center surrounded by a dark reddish border (Fig. 1, B). The spots vary from 2 to 6 mm. in diameter and stand out clearly on the green or reddish green background of the stems and stolons. Frequently, numerous lesions coalesce to form large cankered areas, which may entirely encircle the stem and in later stages cause it to crack. Heavily infected young stems and stolons are frequently killed.

The first symptoms of the disease on young leaves are small brown spots, which subsequently enlarge and become visible from both surfaces of the

[1] The writer wishes to express his appreciation to Dr. R. W. Samson and Dr. R. M. Caldwell for suggestions on the preparation of the manuscript.

[2] Nelson, R. Peppermint diseases and their control. Michigan Muck Farmers Assn. Proc. 18: 44–54. 1936.

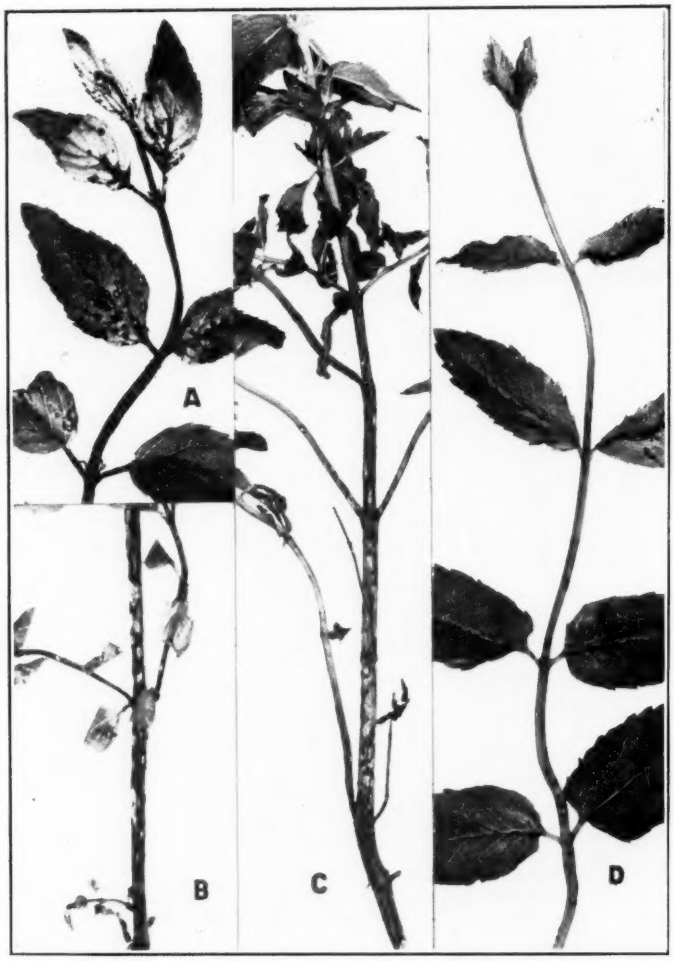

FIG. 1. Symptoms of anthracnose on 2 species of mint. A. Heavy infection on black peppermint shoot; note the constricted stem and yellow terminal leaves. B. Characteristic ash-color lesions with a reddish border on black peppermint stem. C. Lesions on Scotch spearmint stem. D. Lesions on common spearmint stem and leaves 17 days after inoculation. All approximately × 1.

leaf (Fig. 1, A). The spots in later stages are round, approximately 2 mm. in diameter, and light brown with a darker colored margin. Numerous infections on individual leaves may kill large areas of the leaves and cause them to drop prematurely. Frequently, the necrotic areas drop out, giving a shot-hole effect.

The symptoms of the disease on state peppermint, *Mentha piperita* L., white peppermint, *M. piperita* L. var. *officinalis* Sole, and Scotch and common spearmint, *M. spicata* L. (Fig. 1, C and D), are similar to those occurring on black peppermint. On Japanese mint, *M. arvensis* L. var. *piperascens* Malinvaud, the lesions are smaller.

PATHOLOGICAL HISTOLOGY

Stems and leaves of black peppermint with infection in various stages of development were killed in Rawlins' fixative and embedded in paraffin by the n-butyl alcohol procedure outlined by Zirkle.[3] An aqueous solution of anilin blue in picric acid was the most satisfactory stain tried for differentiating the fungus mycelium in the host tissue. A counter stain of orange G was used. The collapsed and dense nature of the contents and walls of the infected cells made it difficult to detect the fungus within the necrotic tissue.

On young stems and leaves the mycelium is, at first, largely superficial or subcuticular. After the epidermal cells are killed, the hyphae penetrate the intercellular spaces of the inner tissues. The cells in contact with and a short distance in advance of the hyphae are killed. Occasionally the infecting hyphae make a limited superficial or subcuticular growth on the leaves before penetrating the underlying tissues. In the section of a young leaf (Fig. 2, B) the infecting hyphae have killed the epidermal cells and then penetrated between the palisade cells. A lesion developing on the ventral side of the leaf usually involves, successively, the epidermis, palisade, spongy parenchyma, and lower epidermis (Fig. 2, A). Frequently the epidermis is sloughed off. Although the spots are limited in size, the continued enlargement of the infected area does not appear to be limited by any visible protective response of the host cells bordering the lesions.

The disease is confined to the epidermis and the cortex of the stems. Affected cells, as in the leaf, die and collapse, to form a slightly depressed lesion (Fig. 2, C). The intercellular mycelium is only sparsely distributed through the necrotic tissues. In the vicinity of the infection, the parenchyma cells of the cortex enlarge, and to a limited extent divide, although no wound phellem is formed. Frequently, a stromatic layer of mycelium is formed on the surface of older lesions from which the conidia are abstricted (Fig. 3, A, B).

ISOLATION AND IDENTIFICATION OF CAUSAL FUNGUS

In the summer of 1933 the causal fungus was isolated from young lesions on the stems and leaves of black peppermint, by the tissue-planting method, and from dilution plates of conidia. The fungus grows slowly and forms a light brown, frequently convoluted type of colony on potato-dextrose agar (Fig. 3, E). In 1936 a fungus similar to that from peppermint was isolated from lesions on the stem of Scotch spearmint. Attempts to isolate the fungus from old lesions were unsuccessful, because of competition of fast-growing secondary organisms in the tissues.

The conidia from cultures are usually slightly larger than those from lesions on the host. Conidia produced on leaf lesions (Fig. 3, C) are hyaline, oblong-elliptical, continuous, sessile on the surface of the superficial, thin stromatic layer and average $6 \times 10\ \mu$ in size with extreme dimensions of

[3] Zirkle, C. The use of n-butyl alcohol in dehydrating woody tissue for paraffin embedding. Science (n. s.) **71**: 103–104. 1930.

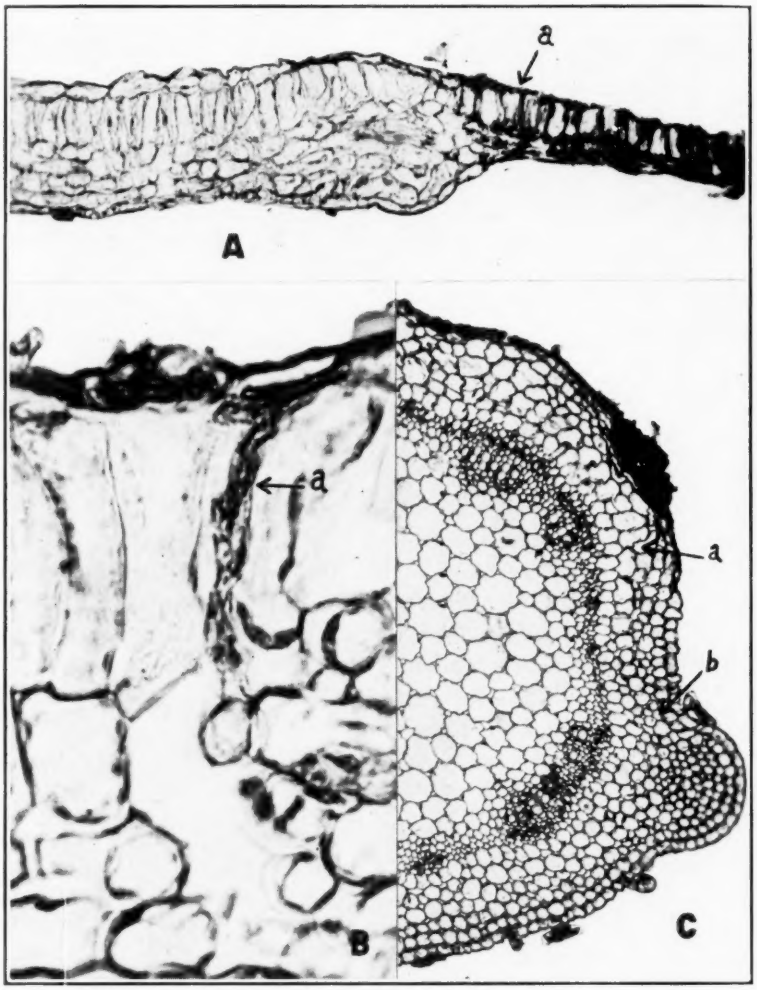

FIG. 2. Cross sections of anthracnose lesions on black peppermint leaves and stem. A. Mature lesion on leaf; note the mycelium of the anthracnose fungus on the ventral surface at a. ×150. B. Incipient infection on leaf, showing hyphae penetrating between the palisade cells at a. The epidermal cells above the penetrating hyphae have been killed. ×670. C. Lesion on young stem. Infected cells are dead. Cortical parenchyma cells at a beneath the lesion have enlarged and divided; compare with normal tissues at b. ×115.

2.8 × 6 μ and 7 × 14 μ. Recently Jenkins[4] described the fungus causing the mint anthracnose disease as *Sphaceloma menthae* Jenkins. She described the fructification as occurring in erumpent, superficial acervuli, 15–80 μ or more in diameter and on a compact, palisade of conidiophores. The writer's observations differ from the above description, in that the conidia were found to be borne on a thin, superficial fertile stroma varying up to 17 μ in thickness (Fig. 3, A, B). The conidia were sessile upon the nonerumpent stromatic layer and no hyphae were observed that could be considered conidiophores.

[4] Jenkins, Anna E. New species of *Sphaceloma* on *Aralia* and *Mentha*. Jour. Washington Acad. Sci. 27: 412–414. 1937.

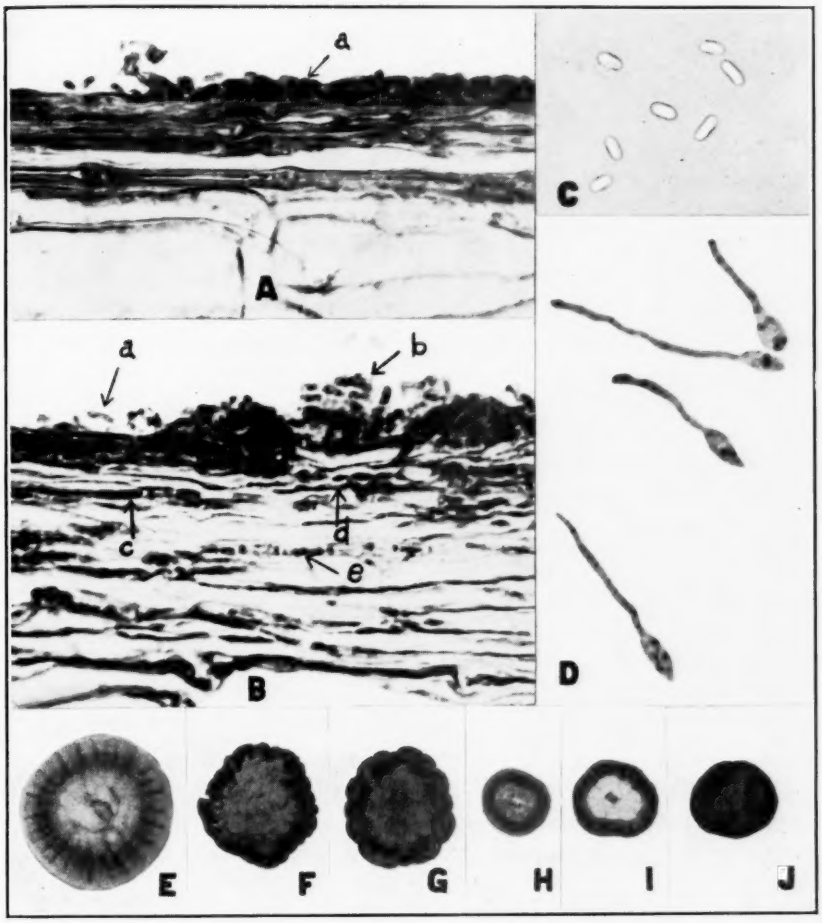

FIG. 3. A. Longitudinal section of stem lesion showing collapsed cells, and superficial stroma at a, from which conidia have been abstricted. B. Longitudinal section of stem lesion with superficial stroma and conidia at a and b, and mycelium among the collapsed cells, at c, d, and e. ×635. C. Hyaline, unicellular conidia. ×490. D. Conidia germinated in distilled water at 80° C. ×640. E–G. Three-week-old cultures on potato-2-per-cent-dextrose, 2 per cent malt-extract, and Leonian's agar media, respectively. ×1. H–J. Three-week-old cultures on 2, 4, and 8 per cent oatmeal agar, respectively. The culture on 8 per cent oatmeal agar sporulated abundantly. ×½.

PATHOGENICITY ON SPECIES OF MINT

The pathogenicity of the cultures was determined by atomizing mint plants in the greenhouse and field with a suspension of spores taken from cultures growing on steamed cornstalks and on oatmeal agar. Plants inoculated in the greenhouse were placed in a moist chamber at 20° C. for 48 hours and then removed to a greenhouse maintained at 21° C. Typical infection resulted on the stems and leaves of black peppermint, following inoculation with suspensions of spores from a culture of the fungus isolated from black peppermint stems and a culture from leaves. Check plants, atomized with

distilled water, remained disease-free. The fungus was reisolated from both leaf and stem lesions of the inoculated plants. Plants of white peppermint and Japanese mint in the greenhouse, and state peppermint in field plots, also were infected by artificial inoculations with a culture isolated from black peppermint.

Later, black peppermint, Scotch and common spearmint plants in pots were inoculated in the greenhouse with cultures of the fungus isolated from Scotch spearmint and black peppermint. After 14 days, all plants inoculated with either culture were heavily infected. The check plants remained free from infection. The fungus was reisolated from each set of infected plants. No difference in the pathogenicity of the cultures was observed.

CULTURAL CHARACTERISTICS OF THE MINT ANTHRACNOSE FUNGUS

Old colonies of the fungus on potato-dextrose agar develop a reddish brown band near the margin and cause the medium to become a light greenish yellow. Since the fungus produces very few conidia on potato-dextrose agar, other media were tried. The fungus grew slowly on sterilized plugs of eggplant fruit, green tomato fruit, tomato stems, green corn stems, and old cornstalks in large test tubes, and formed a brown, warty type of growth that appeared to consist largely of isodiametric thick-wall cells resembling chlamydospores. The cultures on the pith of the sterilized green cornstalks produced an abundance of conidia. On Leonian's medium[5] a grey, heaped type of colony developed, with a dark border and no conidia (Fig. 3, G). Heaped, grey colonies, with a dark border and no conidia, were produced on agar media containing 2, 4, 6, and 8 per cent malt extract, respectively, (Fig. 3, F). On Bacto corn-meal agar and on 0.25, 0.5, and 1 per cent corn-meal agar, the colonies were prostrate, light brown and nonconidial. The fungus was grown also on 0.25, 0.5, 1.0, 2, 4, 6, and 8 per cent oatmeal media, which contained 1.0 per cent Bacto agar. Few conidia were formed by three-week-old colonies on the 1.0 per cent oatmeal medium, but were numerous on 6 and 8 per cent oatmeal media. A thin pinkish colony was formed on media containing 0.25 and 0.5 per cent oatmeal, while on media of higher concentrations of oatmeal the colonies were light colored and brown-marginate (Fig. 3, H to J). When an abundance of conidia was produced, the surface of the colonies became moist and brown.

INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE GROWTH AND SPORULATION OF THE FUNGUS

Duplicate cultures of the fungus were grown in Petri dishes, at 24° C., on both 6 per cent oatmeal agar and potato-2-per-cent-dextrose agar, which had been adjusted to pH values from pH 2 to pH 8.2. The pH adjustments were made by adding 0.1 N HCl or 0.1 N NaOH, to the media after sterilization. The quinhydrone electrode was used.

[5] Leonian's medium consisted of: Dry malt extract, 10 gm.; dextrose, 10 gm.; KNO_3, 2 gm.; KH_2PO_4, 1 gm.; $MgSO_4$, 1 gm.; agar, 20 gm.; distilled water, 1 liter.

The fungus made no growth on the two media at pH 2, but grew well on both when adjusted to pH values between 3.6 and 8.0 (Table 1). In this

TABLE 1.—*Growth and sporulation of Sphaceloma sp. on media of different pH values at 24° C.*

pH value	Oatmeal agar[a]		Potato-dextrose agar[b]	
	Av. diam. of colonies	Sporulation[c]	Av. diam. of colonies	Sporulation
	mm.		*mm.*	
2.0	0	0	0	0
2.5	2	0	9	0
3.1	9	0	7	0
3.6	18	0	...	...
4.1	19	1	26	0
4.6	20	2	30	0
5.0	20	4	32	0
5.5	21	4	33	0
6.0	19	3	32	0
6.5	17	2	30	0
7.0	20	3	33	0
7.5	15	1	35	0
8.0	20	2	33	0
8.2	19	2	...	...

[a] Notes taken after 3 weeks.
[b] Notes taken after 4 weeks.
[c] Degree of sporulation indicated by Arabic numerals 0 to 4; 0 indicating no sporulation, 4 abundant sporulation and intermediate numerals intermediate degrees.

test the limit of tolerance to a low hydrogen-ion concentration was not reached. Sporulation was most abundant on 6 per cent oatmeal agar adjusted to pH 5 and 5.5. Few or no conidia were formed on potato-dextrose agar at any of the pH values used.

INFLUENCE OF TEMPERATURE ON GROWTH AND SPORULATION IN CULTURE

In determining the range of temperature favorable for the growth of the fungus, cultures were grown in duplicate on 1 per cent oatmeal agar at various temperatures between 2° and 33° C. Cultures on 6 per cent oatmeal agar adjusted to pH 5.5 also were placed at temperatures of 15°, 20°, 24°, and 28° C. Five dishes each containing one colony were grown at each temperature. The Petri dish cultures were kept in moist chambers to avoid drying. After 3 weeks, the diameter of the colonies at the different temperatures was measured. The results, as averages at each temperature, are presented in figure 4.

On both oatmeal media the fungus grew well at temperatures between 15° and 28° C., the optimum being at 24° C. At 2° C. the fungus grew very slowly, and at 33° C. no growth occurred. Temperatures of 15° and 20° C. were most favorable for sporulation on the 6 per cent oatmeal agar adjusted to pH 5.5, while little sporulation occurred on the 1 per cent oatmeal agar at any of the temperatures.

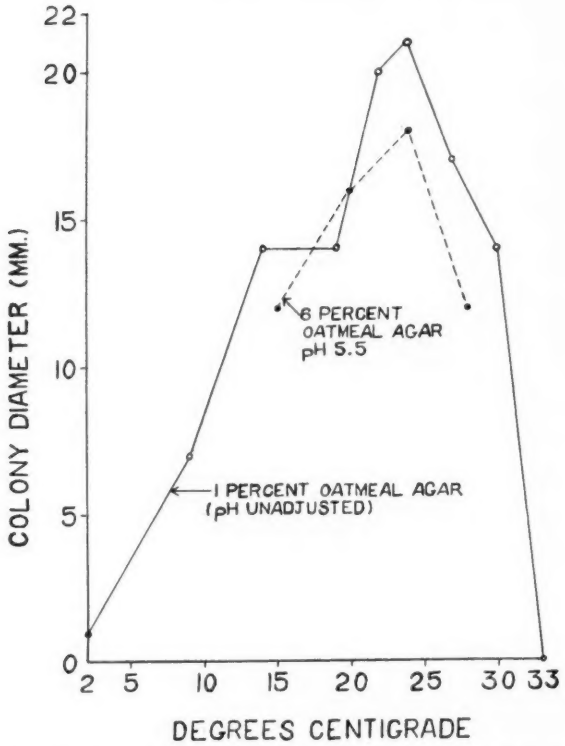

FIG. 4. Influence of temperature on the rate of growth of the mint anthracnose fungus.

OPTIMUM TEMPERATURE FOR SPORE GERMINATION

Germination tests were made in Petri dish moist chambers, each containing one glass slide on which were placed 3 separate drops of a conidial suspension from a 3-week-old culture on 6 per cent oatmeal agar. Two of the dishes, contained in a soil sampling can, were then held at each temperature of the series studied. After 20 hours, toluol was added and germination counts made. Three microscope fields, 1.43 mm. in diameter, were examined per drop, each field containing between 64 and 287 conidia. Between 2200 and 2900 spores were counted at each temperature. No spores germinated at 4.4° and 10° C. At 15.6°, 21°, 26.8°, and 32° C., 51, 88, 93, and 15 per cent, respectively, germinated. The germ tubes formed at 15.6° C. were short and sphaeroid. At 21° C. they were 3 to 6 times the length of the spores while at 26.8° C. they were somewhat shorter (Fig. 3, D), and at 32° C. were very short.

INFLUENCE OF TEMPERATURE ON INFECTION

The relation of air temperature to anthracnose infection was studied with black peppermint plants grown in 4-inch pots. Temperatures of 15.6° C. (60° F.), 21° C. (70° F.), 26.8° C. (80° F.), and 32.1° C. (90° F.) were maintained within a range of 1° C. in illuminated metal chambers. Four

plants atomized with a conidial suspension from a culture, and a check plant sprayed with water were placed at each temperature. High humidity was maintained from water in the bottom of the chambers and by atomizing the plants with distilled water 3 times a day. After 48 hours two pots of inoculated plants were removed from each temperature chamber to a greenhouse maintained at approximately 21° C., while duplicates of these were allowed to remain in the chambers for 14 days.

Seven days after inoculation, infection became evident on the plants removed from the 21° and 26.8° C. chambers after 48 hours and also on the plants held in these chambers during the test. Heavy infection subsequently developed on all the inoculated plants held in the 21° and 26.8° C. chambers for 48 hours or 14 days. Very slight infection occurred on plants held in the 32° C. chamber for 48 hours. No infection occurred on the plants maintained at that temperature for 14 days. Very slight infection developed on plants held in the 15.6° C. chamber for either 48 hours or 14 days. The check plants kept continuously in the chambers remained free from infection in all cases. A repetition of this experiment gave similar results.

OBSERVATIONS ON OVERWINTERING AND DISSEMINATION OF THE FUNGUS

Severely infected peppermint plants growing in 14-quart buckets with bottom drainage were used to study the overwintering of the fungus. On November 6, 1934, the buckets were carefully emptied, disturbing the roots and soil as little as possible. The upper portion of the soil and plants, at a depth of 13 cm. from the surface, was removed intact with a knife. The lower portion of soil and roots was returned to its original position in each bucket. Then the upper section was replaced with the tops down; the tops thus were buried 13 cm. The buckets were then sunk in the soil and remained through the winter in the open. The following April 25, the plants in 11 of the buckets were allowed to develop undisturbed. In another set of 11 buckets, the plants and plant refuse were removed and replanted with disease-free peppermint plants. In 5 buckets the soil was thoroughly stirred, as in cultivation. In 6 more buckets the soil was stirred and covered with old, infected mint refuse. The buckets were divided according to treatment and each set isolated from the other and any source of outside infection, and again sunk in soil.

On May 15, the first infection was evident, and final notes were taken on June 5. At that time abundant infection was present on the mint in the 6 buckets to which the débris had been added and on the plants in 3 of the 5 buckets in which the soil had merely been stirred. The plants in only one of the 11 buckets that were undisturbed, became infected. No infection occurred on the plants in the buckets from which diseased plants and refuse had been removed and then replanted with disease-free plants. From these results it appears that the causal organism overwinters mainly on the old mint refuse rather than in soil free from infected débris and suggests that

new shoots originating from thoroughly buried stolons only rarely become infected beneath the soil.

On June 21, 1934, a number of young mint plants were examined that had been pulled in preparation for replanting a field. The underground portions of the plants were free from any infection, while the aboveground portions of many were heavily infected. A small amount of mint débris was found on the surface of the soil, which had been insufficiently covered in the fall plowing of the field and, apparently, had served as a source of inoculum to the aerial portions. This is interpreted as additional evidence that the disease usually does not reach the surface from infected stolons underground by way of the growing shoots.

Mint débris, bearing lesions of the disease, was collected in the springs of 1935 and 1936 and examined for conidia. Conidia resembling those of the mint-anthracnose fungus were found on the surface of a few of the lesions of the previous season. The fungus was not isolated from dilution plates poured from the spores, nor was an ascogenous stage found.

Observations indicate that the disease usually is introduced into new fields by planting infected plants. Anthracnose frequently has been found uniformly scattered throughout fields of first-year peppermint. In 3 instances the disease was found on the stems and stolons of peppermint plants used for setting new fields.

DISCUSSION

The name, mint anthracnose, has been chosen for this disease in preference to "leopard spot," by which it has come to be known among some mint growers, since the preferred name has been commonly applied to diseases, of this nature, produced on other hosts by fungi of the genus *Sphaceloma*.

Mint anthracnose causes a severe loss of foliage and a cankered condition of the stems, which greatly reduce the vigor of the plants. Peppermint and spearmint oils are secreted by glands, located chiefly on the dorsal surface of the leaves. While no studies have been made of the effect of the disease on yield of oil, it appears certain that such loss of foliage and vigor must greatly reduce it. Weakened plants also may be more susceptible to winter injury. The disease has been observed to be more prevalent on peppermint than on spearmint in the commercial fields. This may be, because peppermint is the more common and more fields of it were observed. In artificial inoculations in the greenhouse, both common and Scotch spearmint were found to be as readily infected as peppermint.

The overwintering of the fungus on infected débris and the introduction of the disease into new fields on infected plants, must be considered in devising means of controlling the disease. Mint fields usually are plowed each fall to protect the plants from cold injury. This practice, if carefully performed to cover all infected mint parts at a depth of 5 to 7 inches, promises to be of value in control of the disease, since the fungus does not appear to

reach the new spring shoots from infected stems and stolons that have been thoroughly covered in the soil.

SUMMARY

Symptoms of mint anthracnose, a recently recognized disease, are described on 3 commercial varieties of peppermint, 2 strains of spearmint, and on Japanese mint. The disease causes defoliation and a severe stunting of the plants, which presumably results in reduced yield of oil.

The causal organism has been recognized as belonging to the genus *Sphaceloma*.

Similar cultures of the causal fungus were isolated from lesions on black peppermint leaves and stems and from Scotch spearmint. Typical infection resulted on the leaves and stems of 3 varieties of peppermint, Japanese mint, and 2 strains of spearmint following artificial inoculations with these cultures.

The fungus is largely intercellular and sparsely distributed in the necrotic tissue. The unicellular conidia of the fungus are sessile on a superficial stroma.

The fungus grows slowly and produces a brown, heaped type of colony on potato-dextrose, malt-extract, and Leonian's agar media, and a slow-growing, prostrate type of colony on corn-meal- and oatmeal-agar media. Conidia are produced copiously on 6 per cent oatmeal agar adjusted to pH 5.5, while few or none are produced on the other media.

The fungus grew well in culture at temperatures between 14° and 28° C., on media adjusted to pH values between 3.6 and 8.2.

Temperatures of 21° to 26.8° C. appeared to be most favorable for infection and the development of the disease.

Overwintering of the fungus was shown to occur on infected mint débris. However, when infected mint débris and living stolons are thoroughly covered with soil they do not appear to be an important source of inoculum to the new shoots in the spring.

The disease is introduced into new fields on infected plants.

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LOCAL VIRUS INFECTIONS IN RELATION TO LEAF EPIDERMAL CELLS

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INTRODUCTION

Local infections develop on leaves of certain plants after they have been wiped with extracts containing the virus of tobacco common mosaic. These infections appear as lesions several days after inoculation and their number is taken as a quantitative index of the virus (3). It has been generally considered that these local infections were caused by the virus, which enters the leaf through trichomes that are injured or broken during the wiping process of inoculation (2, 3). Sheffield (5) found that infections resulted when virus extracts were injected into trichomes by means of a micropipette and has reported (6) that there is no correlation between the number of stomata and local infections when leaves of *Nicotiana glutinosa* L. were wiped with virus extracts.

The writers have recently summarized results that indicate incidental importance of trichomes as centers for local infections when leaves are wiped with virus extracts (1). The complete, and additional, data on the relative importance of stomatal openings are here reported. The purpose of this study was to determine the relative importance of possible ways the virus may enter a leaf as it is wiped. Three possible ways of entry are considered, namely: 1, through wounds in the cells that do not project distinctly from the leaf surface and are referred to in this paper as ordinary epidermal cells; 2, through wounds in cells that project distinctly from the leaf surface and are referred to as trichomes; and 3, through stomatal openings on the leaf surface.

METHODS AND MATERIALS

Several methods were used to evaluate the trichomes as points of entry for the virus from which infection subsequently develops. First, observations were made to determine if any correlation existed between the occurrence of trichomes and evident points of local infection, after leaves had been inoculated by wiping with virus extracts. Second, inoculations were made through mutilated trichomes in one of several ways to simulate inoculations that might result when the whole leaf surface is wiped. These inoculations were so made as to avoid injuring other epidermal cells. The results when inoculations were through only broken trichomes are compared with inoculations of the whole leaf surface by wiping. Third, epidermal areas without trichomes were inoculated. The results, when the epidermis was inoculated without injury to trichomes, are compared with inoculations of the whole epidermis by wiping in the usual manner.

Inoculations to accomplish the comparisons mentioned above were made in several ways. Inoculation of the whole leaf surface was by wiping with

a swab made by tying a small wad of absorbent cotton in a piece of rayon or cheesecloth. This swab was kept saturated by frequently dipping it into the inoculum and thus thoroughly wetting the leaf surface as it was wiped. Inoculations through limited areas or cells of the leaf surface were made under a dissecting microscope. Inoculations through the ordinary cells of the leaf epidermis were made by placing a drop of virus extract on the leaf and rubbing this over the surface with a pinhead padded with rayon cloth. In this way trichomes could be avoided and the inoculations made through the other cells in a manner similar to that when the whole leaf was wiped. Another instrument was made by attaching a short piece of No. 33 constantan wire to a rigid needle. An elbow bent in this flexible wire was very useful to spread the inoculum and very lightly rub epidermal cells while avoiding trichomes. The flexible wire gave a more constant pressure and allowed more freedom of movement and vision under the dissecting microscope than when the pinhead was used.

Inoculations were made by injury to individual trichomes by 3 different methods to simulate injuries that might occur when the whole leaf surface is wiped. One method was to pinch the trichomes off the leaf with a fine-pointed forceps. This was done while the trichomes were partially immersed in the inoculum, thus insuring immediate contact of inoculum and wound. A second method was to cut the trichomes from the leaf while they were partially immersed, thus allowing the inoculum to cover the open stump of the trichome. The trichomes were cut with either of two instruments. A piece of No. 33 constantan wire was attached to a rigid needle and the end of the wire flattened and sharpened to form a small blade. This was satisfactory for cutting in cases where the trichomes were abundant and tender. When the trichomes were more sparse and tough, a larger instrument was used, made by cementing a very small chip from the edge of a safety-razor blade to a rigid wire needle. A third method of inoculating the trichomes was to break or bruise them by use of a glass rod drawn to a fine end and rounded in a flame. An elbow bent in the flexible wire instrument described above was also satisfactory for breaking and bruising the trichomes. These instruments had no sharp edges, thus they left small wounds in comparison to the larger more open wounds made by cutting as above. In all cases, when inoculating individual trichomes, care was taken not to injure other cells of the epidermis. In no case was the inoculum allowed to dry on the leaf surface before being rinsed away by water.

The inoculum was prepared by macerating and pressing the juice from leaves of mosaic-diseased tobacco plants. This was used either undiluted or in dilutions no greater than two times.

In these studies several combinations of plant species and viruses were used. Common mosaic of tobacco was used on leaves of *Nicotiana glutinosa* L., *N. rustica* L., and pepper, *Capsicum frutescens* L. A yellow-mosaic mutant of common mosaic, Type A previously described by McKinney (4) was used on leaves of *N. sylvestris* Spegaz. and Comes. Local infections were

produced by these viruses on the leaves of the respective plants. Leaves of *N. sylvestris* were used in most of the studies as a type that has numerous tender trichomes, which are readily mutilated as the leaf is lightly wiped. *N. rustica* and *N. glutinosa* are comparable to *N. sylvestris* in this respect. In contrast to the above, leaves of pepper were used as a type that has few trichomes and these are much tougher and less readily mutilated. The number of trichomes per square centimeter of leaf surface varies on the same leaf and between leaves. Sample counts to estimate an average number of trichomes per square centimeter on leaves of each of the above species show a distinct difference between the leaves of the *Nicotiana* species and the pepper. *N. sylvestris* had an average of 346 trichomes per square centimeter; *N. glutinosa*, 165; *N. rustica*, 150; and pepper, 16. The epidermis of the pepper leaf is also much tougher than that on the leaves of the *Nicotiana* species. The pepper leaves were also very satisfactory for determining the relationship between stomata and the occurrence of local lesions.

RELATION OF TRICHOMES TO LOCAL INFECTIONS

Leaves that had been inoculated by wiping with virus extract were examined to determine any relationships that might exist between the location of broken trichomes and the local lesions. The lesions were classified into 3 groups: 1, those with a trichome at or near the center; 2, those with a trichome at or near the periphery; and 3, those void of trichomes. The results of these observations are given in table 1. On the pepper leaves, which have comparatively few trichomes, 82.4 per cent of the lesions showed no relation whatever to trichomes. In comparison, on the leaves of *Nicotiana sylvestris*, which have 20 to 30 times as many trichomes per unit of area, 39.9 per cent of the lesions showed no relation to trichomes. Thus the percentage of lesions that have no relation to trichomes, decreases as the number of trichomes increases. This decrease indicates a casual rather than a causal relation between accomplishment of infections and breaking trichomes, and that infection occurs through cells of the epidermis other than trichomes when the leaves are wiped.

TABLE 1.—*Location of trichomes in relation to lesions on leaves wiped with virus inoculum*

Test plant	Relation of trichomes to lesions					
	Center		Periphery		None	
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Nicotiana sylvestris	124	39.9	63	20.2	124	39.9
Pepper	38	16.3	3	1.3	192	82.4

Tests were carried out to determine if the number of lesions is greatly reduced on leaves that have had most of their trichomes destroyed before inoculation. The right half of each *Nicotiana sylvestris* leaf was wiped

with distilled water in the manner usual for inoculation. This first wiping destroyed about 95 per cent of the trichomes, and there was very little evidence that other cells of the epidermis had been severely injured. Such leaves were allowed to stand several days before inoculation of the whole leaf by wiping with virus extract. It was found that the number of necrotic lesions was reduced only 6.8 per cent on the right halves of the leaves that were wiped twice in comparison with the left halves of the leaves, which were wiped only at the time of inoculation.

The above test was repeated, except that fine carborundum dust and distilled water were used to rub the right halves of the leaves several days before inoculation. This first wiping destroyed about 98 per cent of the trichomes, and many ordinary cells of the epidermis were severely injured or killed. After inoculation it was found that the number of necrotic lesions was reduced 31 per cent on the twice-wiped right halves in comparison with the left halves, which were wiped only at the time of inoculation (Table 2).

TABLE 2.—*Reduction in the number of local infections on leaves of Nicotiana sylvestris due to destruction of trichomes and to severe injury to other epidermal cells by wiping the right half of each leaf two to six days before inoculation by wiping the entire leaf*

Leaves tested	Lesions on the half leaves with no mutilated cells before inoculation	Lesions on the half leaves with trichomes mutilated before inoculation	Lesions on the half leaves with trichomes and other epidermal cells severely injured before inoculation[a]	Reduction in number of lesions due to mutilation of cells before inoculation
No.	*No.*	*No.*	*No.*	*Per cent*
30	1039	965[b]	.....	7.1
30	905	.....	568	37.2
17	1334	1247[b]	.....	6.5
13	1008	.....	759	24.7
3	160	160[b]	.....	0.0
1	72	60[c]	.....	16.7

[a] Rubbed the right half of each leaf with virus-free water and fine carborundum dust to destroy about 98 per cent of the trichomes and severely injure other epidermal cells.

[b] Wiped the right half of each leaf with virus-free water to destroy about 95 per cent of the trichomes without evident severe injury to other cells of the epidermis.

[c] A wire blade was used to mutilate the trichomes without injury to other cells.

A smaller number of local infections occurred on the halves of the leaves that had been wiped or rubbed with water several days before the inoculation with virus extract. This is considered to be the result of destruction or severe injury to cells through which infection would normally occur on the uninjured leaf. The relative importance of trichomes and ordinary epidermal cells as points through which infections normally occur as the leaves are wiped with virus extract, is indicated by these data. Destruction of about 95 per cent of the trichomes several days before inoculation, reduced the number of local infections an average of 6.8 per cent. In comparison, destruction or severe injury to some of the ordinary epidermal cells reduced the number of local infections an average of 24.2 per cent.

Comparative tests were made with both young and old leaves of *Nicotiana rustica* and *N. glutinosa* in the first stages of senility. The number of trichomes per unit of area on the youngest leaves of these species is more than 3 times that on the old leaves. On these old leaves with fewer trichomes, the average number of lesions per unit area was from 1.5 to 9.6 times greater when inoculations were made by wiping the leaves with virus extracts diluted to 100X in water. Thus it is apparent that the number of lesions is not necessarily proportional to the number of trichomes per unit of area.

INOCULATIONS THROUGH TRICHOMES

Inoculations were made through individual trichomes by cutting or pinching counted numbers of them from the leaves under a dissecting microscope. The inoculations were made at random over the leaf, and observations were made at subsequent intervals under a dissecting microscope to check the location and number of infections with the points of inoculation. The data presented in table 3 show that infection developed from a small percentage of the inoculations through the mutilated trichomes.

TABLE 3.—*Percentages of infection that resulted when inoculations were made by mutilation of individual trichomes on leaves of Nicotiana species and pepper*

Test plant	Method of inoculation	Trichomes inoculated	Local infections	
		No.	*No.*	*Per cent*
N. sylvestris	Pinching	730	4	0.5
" "	Cutting	1500[a]	30	2.0
" "	"	1420[b]	33	2.3
N. rustica	"	30	1	3.3
Pepper	"	870	0	0.0

[a] Large trichomes.
[b] Small trichomes.

It will be noted that none of the 870 trichomes cut from the pepper leaves served as points of infection. In other tests some infections have resulted on pepper leaves when the trichomes were cut, but no accurate counts were made of the trichomes so inoculated. It is estimated that infection has developed from less than 0.5 per cent of the inoculations on pepper leaves by this method. No significant difference appeared between the percentage of infections that resulted from inoculation through either the very large or very small trichomes on the leaves of *Nicotiana sylvestris*. Pinching the trichomes from the leaves of *N. sylvestris* accomplished a lower percentage of infection than did cutting them. It is suspected this may be due in part to the tendency of the forceps to pinch and close the top of the trichome as it was torn from the leaf. These trichomes may be compared to those that are bent and compressed against the leaf as the surface is wiped.

Inoculations were made by mutilating all the trichomes in small areas on leaves of *Nicotiana sylvestris*, *N. glutinosa*, and *N. rustica* without evident injury to the ordinary cells of the epidermis. These areas of mutilated

trichomes were scattered over the right half of each of the leaves. The opposite halves of these leaves were inoculated by wiping the entire surface in the usual manner. The results of these inoculations are computed on a basis of the number of local infections per square centimeter and presented for comparison in table 4. When the points of entrance for the virus were

TABLE 4.—*Comparative number of local infections when leaves were wiped and when the trichomes only were inoculated*

Test plant	Control inoculation by wiping entire half leaves		Inoculation through mutilated trichomes in small areas			
	Av. infections per cm.²		Size of area	Areas[a] inoculated	Average infections per cm.²	
	No.	*Per cent*[b]	*Cm.²*	*No.*	*No.*	*Per cent*
Nicotiana sylvestris	6.0	100	.196	15[c]	2.12	35.3
N. glutinosa	11.47	100	.38	128[d]	2.57	22.4
N. rustica	4.59	100	.38	142[d]	0.55	12.0

[a] All the trichomes on these areas were inoculated.
[b] The control inoculation is considered 100 per cent in each instance for comparison with test inoculations.
[c] Trichomes were mutilated with wire blade.
[d] Trichomes were broken and bruised with fine glass instrument.

limited to broken or injured trichomes, the number of infections per unit of leaf area was much less than when the entire surface of the leaf was wiped with the virus extract. Infections developed at a small percentage of the mutilated trichomes and these infections represent a small percentage of the total number obtained by wiping the leaves.

It is possible that an injury sufficient to break a trichome is a too severe shock to the cell; thus the chances of its serving as a point of entry and establishment of the virus may be reduced. Degrees of injury to a living cell cannot be accurately evaluated. The trichomes of the species of *Nicotiana* studied were so tender that it was difficult to injure them with the instrument described without causing their complete collapse. On the pepper leaves, however, this was not true. As has been stated, cutting the trichomes from the pepper leaves gave a very small percentage of infection. Inoculations were made by bruising or breaking the trichomes on these leaves, as described above, with the elbow of fine flexible wire. However, no accurate data were obtained, since injury to ordinary epidermal cells could not be avoided with certainty. It, nevertheless, is pertinent that approximately 5 times more infections resulted from bruising than from cutting the trichomes. Severe injury, such as might occur to cells if too much pressure is used when leaves are inoculated by wiping, would not be so effective as a medium amount of pressure sufficient to slightly wound the cells.

Wisconsin-Havana seed tobacco plants were inoculated by mutilating the trichomes on 0.2 sq. cm. of a leaf on each plant. When the inoculated leaf was $5 \times 2\frac{1}{2}$ inches or larger, 49 per cent of 49 inoculations were positive and

symptoms appeared in an average of 16 days. In comparison, when the younger leaves, less than $5 \times 2\frac{1}{2}$ inches, were inoculated, 82 per cent of 66 inoculations were positive and symptoms appeared in an average of 10 days. The older leaves were not so susceptible to inoculation through the trichomes and required a longer period for development of symptoms. Inoculations into the stems of 24 plants were 100 per cent positive and required an average of 10 days for development of symptoms.

INOCULATION OF EPIDERMAL AREAS WITHOUT TRICHOMES

Since the trichomes are comparatively sparse in the epidermis of pepper leaves, they could be avoided while inoculating small areas between them. These areas were inoculated under a dissecting microscope by lightly rubbing the virus extract over them with a padded pinhead or with an elbow bent in a fine, flexible wire. The irregularity in size and shape of the areas between the trichomes did not allow their accurate measurement, but it is estimated that contact was made with approximately one-half of the entire leaf area when the padded pinhead was used. The flexible wire elbow allowed better vision and greater freedom of movement under the microscope, and made possible an effective contact with approximately two-thirds of the entire leaf area. In table 5 the results of such inoculations are compared with the results when comparable leaves were inoculated by wiping in the usual manner. On these pepper leaves the number of lesions that developed after the areas between the trichomes were inoculated is comparable to the number obtained by wiping in the usual manner. These data show that when pepper leaves are wiped most of the inoculations occur through ordinary epidermal cells rather than trichomes.

TABLE 5.—*Comparative numbers of local lesions on pepper leaves after the leaf surface was wiped with a cloth swab and after the epidermis was rubbed with a flexible wire elbow or padded pinhead in a manner to avoid striking the trichomes*

Method of inoculation	Leaves inoculated	Leaf area contacted	Average infections per leaf
	No.	*Per cent*	*No.*
Rubbed with flexible wire elbow	6	67[a]	15
Control—wiped	3	100	22
Rubbed with padded pinhead	5	50[a]	7
Control—wiped	2	100	20

[a] Estimated percentage of total leaf area touched as inoculations were made to avoid trichomes.

Since cells of the epidermis other than the trichomes are inoculated when leaves are wiped with virus extract, some observations were made to determine the injury to these by the wiping. Pepper leaves were wiped with distilled water, after which the plants were placed in a chamber at 88° F. Leaves were then left in these chambers about 24 hours and collected in the morning before starch had accumulated from photosynthesis. They were immediately decolorized in hot acetic alcohol and stained in an iodine potassium iodide solution to determine the presence of starch. Examination

showed that the starch had been retained in severely injured cells. Such cells were not of great enough number to account for more than a small fraction of the local infections expected on such leaves if they had been wiped with an undiluted virus extract. Epidermal cells at the base of the trichomes frequently retained their starch. This was particularly true of the cells on the side of the trichomes where the acute angle had been formed with the epidermis as the leaves were wiped. Injuries less severe than were apparent under a magnification of 28 times are necessary to account for the largest part of the local infections that occur when pepper leaves are wiped with undiluted virus extract.

STOMATA IN RELATION TO LOCAL INFECTIONS

Leaves of the pepper used in these studies have very few trichomes on either the upper or lower epidermis. The number of stomata in the lower epidermis is 593 per sq. mm. and in the upper epidermis 26 per sq. mm., as determined by counts of sample areas of each. Forty-six leaves were wiped with fresh extracts containing the virus of tobacco common mosaic. In the case of each leaf one-half was wiped on the upper side and the other half, on the lower side.

Actual counts were made and it was found that 6.04 and 7.18 lesions per sq. cm. appeared on the halves wiped on the lower and upper sides, respectively. Thus it seems evident that stomata are not important loci for local necrotic lesions when the leaves are inoculated by wiping. These observations are in agreement with those made by Sheffield (6) on *Nicotiana glutinosa*. He states that the difference between the number of lesions when the upper and lower leaf surfaces are inoculated, "is probably due to there being fewer trichomes on the lower surface." However, it seems more probable that some factors other than the number of trichomes cause this difference in number of lesions.

DISCUSSION

From the observation of lesions on leaves of pepper and *Nicotiana sylvestris*, which had been inoculated by wiping, it is apparent that the greater the number of trichomes per unit of leaf area, the greater the chance that a lesion and trichome be coincident. The proportion of lesions that are coincident to injured trichomes is not in exact agreement with the relative numbers of trichomes per unit of leaf surface. For example, the leaves of *N. sylvestris* have approximately twenty times more trichomes per unit of area than the pepper but the proportion of lesions coincident to injured trichomes is only about twice as great. This is considered due to a factor of tenderness or vulnerability of the trichomes. As trichomes are inoculated separately or when whole leaves are wiped with virus extract, the relative degrees of injury cannot be accurately evaluated. However, cutting trichomes from pepper leaves, as compared to inoculation by bruising the trichomes, gave fewer infections. In the tests described in table 4 when attempt was made to inoculate by bruising the trichomes on *N. glutinosa* and *N. rustica* leaves, more trichomes were severely injured on the leaves of the latter and fewer lesions

were obtained. Thus tenderness and the resulting severe injury to a trichome may reduce the chance for accomplishment of infection at that point.

Small areas on the leaves of *Nicotiana sylvestris* were inoculated by mutilation of all the trichomes. The number of lesions per sq. cm. obtained on these areas was 35.3 per cent of the number obtained by wiping the opposite half leaf. This is consistent with the 39.87 per cent of the total lesions observed to have trichomes in their centers on wiped leaves of this species. These lesions are considered to be the result of inoculation through a broken trichome. In view of the above, it seems probable that 20.26 per cent of the lesions on wiped leaves observed with a broken trichome in their periphery are the result of inoculations through epidermal cells near the trichomes.

SUMMARY

When leaves were wiped with virus extracts the coincidence of broken trichomes and local infections seemed to be largely a matter of chance and the number of lesions was not necessarily proportional to the number of trichomes per unit of area.

There was a much greater reduction in the number of lesions obtained when both the trichomes and other epidermal cells were injured several days prior to inoculation by wiping the leaf than when injury previous to inoculation was limited to the trichomes.

Inoculations by mutilating the trichomes with special instruments without injury to other cells of the epidermis obtained a smaller number of lesions per sq. cm. than when inoculations were made by wiping the leaf surface.

The number of lesions per unit of area inoculated was not significantly different when either the whole surface of pepper leaves was wiped or limited areas without trichomes were rubbed.

Inoculations made by wiping the upper and lower surfaces of pepper leaves did not result in significantly different numbers of lesions. Since there are over 20 times as many stomata in the lower epidermis, it seems evident that they are not important loci for local infections.

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EXPERIMENTS WITH APHIDS AS VECTORS OF TULIP BREAKING[1]

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Investigations of tulip mosaic, commonly known as breaking, at the John Innes Horticultural Institution in England (2, 5, 6) and at the Oregon Agricultural Experiment Station (3, 4) have proved it to be a transmissible virosis. The history of the disease has been treated in detail by McKay and Warner (4), and McWhorter (7, 8) has recently shown that two viruses are concerned in breaking. In the typical broken tulip the two are present in balanced proportions. Aphid transmission has been reported by McKay and associates (3, 4) in Oregon, and by McKenny Hughes (5, 6) in England. This paper presents the Oregon data on aphid transmission.

MATERIALS AND METHODS

Transmission tests were carried out on tulips growing in Dutch beds in the field. The type of insect cage used covered 2 rows of 10 tulips each. The control series for each set of inoculated plants consisted of "sister bulbs," as explained in a previous paper (1). Several types of transmission tests were employed: (a) Aphids were placed on broken tulips for 13 to 29 days, then transferred to caged healthy tulips for the balance of the season; (b) colonies were developed on broken tulips in pots and a potted plant, with the insects on it, was set in each cage; (c) nonviruliferous aphids were introduced into cages in which both healthy and broken tulips were growing; and, in addition, a few cages enclosing healthy and broken tulips became naturally infested with *Macrosiphum* (*Illinoia*) *solanifolii* (Ashm.).[4] These several types of tests are indicated in tables 1 and 2.

The aphid colonies, except the colony of *Anuraphis tulipae* (Boyer), used in the 1928 and 1929 transfer series, were pure lines developed from single individuals and were proved nonviruliferous before the start of the experiments by previously feeding on healthy tulips. Mass cultures from colonies on broken tulips were used in the 1926–27 and 1927–28 tests.

FIELD TRIALS WITH APHIDS AS VECTORS

In a test conducted by the junior author in 1926, breaking was transmitted by aphids reported (3) as *Myzus solani* (Kalt.). Specimens of this colony are no longer available, and, unfortunately, no record remains of the

[1] Cooperative project of the Oregon Agricultural Experiment Station and the Bureau of Plant Industry, U. S. Department of Agriculture; experimental work conducted at Corvallis, Oreg.

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[4] All determinations of aphid species were made by Dr. P. W. Mason.

authority responsible for the identification. Subsequent tests have failed to confirm *M. solani* as a vector.

The results of 3 seasons' transmission tests[5] are shown in tables 1 and 2. Breaking never was detected during the season of inoculation; therefore, all records of transmission were made in the year following aphid exposure. Corresponding "sister bulb" (1) controls remained healthy during the following season in all experiments tabulated.

The data in tables 1 and 2 show *Macrosiphum (Illinoia) solanifolii* transmitted breaking in 12 of 28 tests, 60 of 419 plants exposed showed the disease, or 14.3 per cent. Similarly, *Myzus persicae* transmitted breaking in 7 of 10 tests, and 34 of 196 plants, or 17.4 per cent were infected. *M. solani, Anuraphis tulipae*, and *Rhopalosiphoninus tulipaella* failed to transmit breaking in any test; the numbers of plants exposed were respectively 117, 40, and 77. *M. circumflexus* apparently transmitted breaking in 1 of 5 tests, 3 of 95 plants exposed showing the disease, or 3.2 per cent. No evidence of mixture of this colony with a recognized vector species was detected on examination during the course of the test, and no other source of error in the test appears likely.

Manual transfer of supposedly viruliferous aphids was followed by erratic development of tulip breaking. Similar results were obtained in transmission studies of iris mosaic (1). The data for *Macrosiphum (Illinoia) solanifolii* and *Myzus persicae* in tables 1 and 2 show 11 of 27 tests by this method were successful, and 46 of 517 plants exposed or 8.9 per cent became infected. Some tests were highly successful (*e.g.*, nos. 53, 55, 57, 350); in these the aphids were introduced on diseased leaves, rather than brushed from them. Introducing the vector on a potted Rembrandt tulip produced infection in 4 of 5 tests and in 20 of 100 plants exposed. Irregularity in performance here may be due to differences in rate of aphid migration from the diseased plant. Nonviruliferous aphids placed in cages containing both broken and healthy tulips spread the disease in 2 of 4 tests and infected 6 of 71 plants exposed (8.5 per cent). In 2 tests that failed (nos. 332, 332a) the aphids were introduced late in the season (May 22). When broken and healthy tulips, caged together, became naturally infested with *M. (I.) solanifolii* (nos. 165, 109), 22 of 27 plants or 81.5 per cent became infected. Natural infestation presumably results in extensive spread of breaking only when the aphids become established on diseased plants early in the season. These colonies were detected April 30 and may have been present earlier.

Natural infestation by *Macrosiphum (Illinoia) solanifolii* of 19 cages enclosing 364 healthy tulips in the spring of 1928 induced no breaking in the progeny of these plants in 1929. This indicates that the potato aphid arrived at the bulb plot in a nonviruliferous condition. In the same season 85 healthy tulips were grown under cages together with broken tulips in the absence of aphids; none were broken in the following year. The way in which

[5] The authors are indebted to Dr. F. P. McWhorter for taking the records in the spring of 1930.

TABLE 1.—*Transmission of tulip breaking by aphids. Trials with Macrosiphum (Illinoia) solanifolii*

Trial number	Broken variety used as source of inoculum	Date of transfer[a]	Healthy variety exposed	Plants exposed and surviving in following season	
				(*Total number*)	(*Per cent infected*)
		1927 transfers—1928 results			
29	Farncombe Sanders Rembrandt	4/13/27	Clara Butt	19	5.2
2	Clara Butt #29	5/10/27	King Harold	18	5.5
5	" " "	"	Allard Pierson	19	0
8	" " "	"	Pride of Haarlem	18	0
11	" " "	"	William Copland	20	0
53	Golden Bronze	"	Clara Butt	18	38.8
54	Jupiter	"	"	19	10.5
55	Godet Parfait	"	"	18	50
56	Dr. Hardy	"	"	19	21
57	Edmee	"	"	19	31.5
		1928 transfers—1929 results			
165	King Harold	4/30/28[d]	King Harold	17	70
109	Farncombe Sanders Rembrandt	5/1/28[d]	Clara Butt	10	100
70	" " "	5/10/28	"	20	0
76	" " "	"	"	20	0
77	" " "	"	"	19	0
72	Bybloem Violet Gloriosum	"	"	19	0
73	Bizarre Zebra	"	"	19	0
157	Farncombe Sanders Rembrandt	5/23/28	Farncombe Sanders	17	0
248	" " "	"	Princess Elisabeth	20	0
224	Remembrance Rembrandt	"	Remembrance	20	0
		1929 transfers—1930 results			
266	Farncombe Sanders Rembrandt	4/20/29	Clara Butt	20	0
270	" " "	4/26/29[b]	"	20	20
274	" " "	4/26/29[b]	"	20	5
310	Remembrance Rembrandt	5/3/29[c]	Remembrance	16	19
347	Farncombe Sanders Rembrandt	5/6/29	Mrs. Moon	19	0
352	" " "	5/7/29	Bouton d'Or	20	0
332	Inglescombe Yellow	5/22/28[c]	Inglescombe Yellow	19	0
332a	" "	5/22/28[c]	"	17	0

[a] Manual transfer unless otherwise noted.
[b] Aphids introduced on broken tulip in a pot.
[c] Broken and healthy tulips caged together and nonviruliferous aphids added.
[d] Broken and healthy tulips caged together and naturally infested by aphids.

TABLE 2.—*Transmission of tulip breaking by aphids. Trials with Myzus persicae (Sulz.), M. circumflexus (Buckt.), M. solani, Anuraphis tulipae, and Rhopalosiphoninus tulipaella (Theob.)*

Trial number	Broken variety used as source of inoculum	Date of transfer[a]	Healthy variety exposed	Plants exposed and surviving in following season (*Total number*)	(*Per cent infected*)
			Myzus persicae		
33	Centenaire	4/13/27	Clara Butt	18	5.5
36	Rembrandt	4/27/27	"	20	10
37	"	3/20/28[b]	"	20	60
67	Farncombe Sanders Rembrandt	5/10/28	"	20	0
99	" " "	"	Pride of Haarlem	20	0
272	" " "	4/26/29[b]	Clara Butt	20	0
278	" " "	4/27/29[b]	"	20	15
350	" " "	5/15/29	Bouton d'Or	19	58
331	Inglescombe Yellow	5/31/29[c]	Inglescombe Yellow	19	15.8
			Myzus circumflexus		
68	Farncombe Sanders Rembrandt	5/10/28	Clara Butt	19	0
269	Clara Butt	4/26/29[c]	"	18	0
273	Farncombe Sanders Rembrandt	4/26/29[b]	"	20	0
328	Princess Elisabeth	5/3/29[c]	Princess Elisabeth	18	16.7
351	Farncombe Sanders Rembrandt	5/7/29	Bouton d'Or	20	0
			Myzus solani		
69	Farncombe Sanders Rembrandt	5/10/28	Clara Butt	20	0
182	" " "	5/11/28	William Pitt	20	0
268	" " "	4/26/29[b]	Clara Butt	20	0
275	" " "	4/26/29[b]	"	20	0
346	" " "	5/26/29	Mrs. Moon	19	0
330	Inglescombe Yellow	5/3/29[c]	Inglescombe Yellow	18	0
			Anuraphis tulipae		
71	Farncombe Sanders Rembrandt	5/10/28	Clara Butt	20	0
354	" " "	5/7/29	White Queen	20	0
			Rhopalosiphoninus tulipaella		
66	Farncombe Sanders Rembrandt	5/11/28	Clara Butt	20	0
271	" " "	4/26/29[b]	" "	20	0
349	" " "	5/26/29	Bouton d'Or	18	0
279	Clara Butt	5/3/29[c]	Clara Butt	19	0

[a] Manual transfer unless otherwise noted.
[b] Aphids introduced on broken tulips in pot.
[c] Broken and healthy tulips caged together and nonviruliferous aphids added.

caged plants became naturally infested with aphids was not determined, as the cages were apparently aphid-tight. In 1928 they were set in place over the tulips on February 26 to March 16 while the plants were small and inspection failed to reveal any aphids present at the time. One lot (no. 109), containing 10 healthy and 8 broken tulips, was caged January 30 before the plants had emerged; contamination with *M.* (*I.*) *solanifolii* occurred and 100 per cent transmission resulted. In the spring of 1929 identical cages were placed over tulips on March 14, and all were fumigated in place on April 2 and 3 with calcium cyanide—½ ounce per 1000 cubic feet for 4 hours—under a heavy canvas cover at a temperature of about 65° F. No natural infestations occurred within these fumigated cages.

There is some indication in the data of tables 1 and 2 that early infestation is more effective than late infestation in transmission of breaking. Three inoculations with *Macrosiphum* (*Illinoia*) *solanifolii* and *Myzus persicae* on April 15 were successful; 6 of 8 between April 16 and April 30; 10 of 22 between May 1 and May 15, but none of 5 after May 16 proved effective.

Four attempts to transfer iris mosaic to tulip by *Macrosiphum* (*Illinoia*) *solanifolii* failed. Similarly, attempts to transmit a virus to tulip from symptomless Bliss Triumph potato, and from Bliss Triumph affected with mild mosaic, crinkle, leaf roll, and rugose mosaic by *Myzus persicae* also failed. *M. circumflexus* failed to transmit lily mosaic to tulip in a single trial.

APHIDS ON TULIP BULBS IN STORAGE

A series of tests was conducted to determine whether the aphids that feed on tulip bulbs in storage are capable of transmitting tulip mosaic from bulb to bulb. Colonies of the aphids were allowed to feed on healthy tulip bulbs, transferred to bulbs of Rembrandt tulips for 17 to 26 days, then to healthy bulbs of Clara Butt for 17 to 38 days. The latter were fumigated and grown in the field for 2 seasons following these tests. All remained healthy. Forty-nine healthy tulips survived in the field after such experimental feeding of *Rhopalosiphoninus tulipaella,* 47 after such trials with *Anuraphis tulipae,* 20 after exposure to *Myzus* sp., and 20 after such a trial with an undetermined species. *Myzus persicae* found feeding on tulip bulbs in the laboratory in October was maintained on bulbs until February 15, but tests of virus transfer were not completed.

APHIDS OCCURRING ON TULIPS

Forty collections of aphids from tulip leaves identified by P. W. Mason include *Macrosiphum* (*Illinoia*) *solanifolii* 31, *Myzus persicae* 6, and *Myzus* sp. 3. On stored tulip bulbs *Rhopalosiphoninus tulipaella* was taken twice, *M. persicae* once, and an undetermined species once. *Macrosiphum* was found on tulips in Oregon in April and May (once on June 13), in Washington in May, and in California in late February and early March. The recognized vectors of tulip breaking appear to be common on tulips in nature, while the possible vectors *M. solani* and *M. circumflexus* are not represented in this series of collections from tulips.

DISCUSSION AND CONCLUSIONS

McWhorter (7, 8) has shown that the average broken tulip results from infection with 2 viruses in balanced proportions. Tulip Virus 1 or "color-removing," when predominating in the mixture, produces an accentuated white break; Tulip Virus 2 or "color-adding," when nearly pure, produces "red break" or "self break." McKenny Hughes (5, 6) has noted white break and red break in plants infected by means of aphids. In our experiments the usual result of aphid transmission was an average break, but some segregation of inoculated clumps into average and self break occurred. It is clear that the vectors, *Myzus persicae* and *Macrosiphum* (*Illinoia*) *solanifolii*, are each able to carry both of the tulip viruses, and that they are not selective for either under the conditions of our experiments.

McKenny Hughes (5, 6) found breaking appeared in the same season the tulips were inoculated, 6 weeks or less after viruliferous *Myzus persicae* or *Macrosiphum gei* (Koch) had been allowed to feed. Such current-season effects never appeared in our work, whether inoculations were made by aphids or by mechanical methods.

The present results are in accord with those of McKenny Hughes (5, 6) in establishing *Macrosiphum* (*Illinoia*) *solanifolii* (*Macrosiphum gei* of that author) and *Myzus persicae* as the principal vectors of tulip breaking. McKenny Hughes (6) has reported *Anuraphis tulipae* a definite vector of breaking in storage. We were unable to gather any evidence from our tests that this species could act as a vector, either in storage or on growing plants. On the other hand, we have an isolated record, apparently reliable, in which *Myzus circumflexus* transmitted breaking, and a report previously published (3, 4), but resting on incomplete records, of transmission by *M. solani*. The 2 species last named are not commonly found on tulips, although they fed on them readily under the conditions of our tests.

The suggestion based on the present data that late infestation did not serve to introduce the viruses of breaking into the bulbs is more clearly shown in McKenny Hughes' (6) data. The factors affecting transmission of viruses by aphids have been studied in detail by others, notably by Watson (9), since the present work was completed.

SUMMARY

Experiments at the Oregon Agricultural Experiment Station from 1926 to 1930 show that *Myzus persicae* and *Macrosiphum* (*Illinoia*) *solanifolii* are vectors of breaking in tulips.

Both these species of aphids are vectors for both the viruses of breaking distinguished by McWhorter.

Myzus circumflexus apparently transmitted breaking in one trial. No evidence was obtained to support our earlier report of transmission by *M. solani*. No evidence appeared in our tests that the bulb-infesting species *Anuraphis tulipae* or *Rhopalosiphoninus tulipaella* can transmit breaking.

No symptoms appeared during the season of inoculation in any of our experiments.

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INACTIVATION OF TOBACCO-MOSAIC VIRUS IN CURED TOBACCO LEAVES BY DRY HEAT[1]

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Dry-thermal inactivation of tobacco-mosaic virus in air-cured White Burley tobacco, apparently not reported in the literature, was determined for a series of temperatures with infectious 1882 White Burley tobacco and White Burley tobacco cured in 1936 containing 3 strains of virus: yellow, green, and burning. The purpose of this paper is to report these data.

Tobacco-mosaic virus in cured tobacco that had been kept 52 years in a box, in a dry barn, was found (4) to have retained some virulence. Mosaic leaf material, dried before exposure to 140° C. for 0.5 hour, has been reported (1) noninfectious, while that exposed for the same period at 120° C. remained infectious. Virus in expressed plant juices or aqueous dilutions of these juices (5) is inactivated at a lower temperature, nondiluted plant juice at 75° C. to 96° C. for 40 days to 1 minute, and juice diluted 1:20 in water at 68° C. to 92° C. for 20 days to 1 minute, respectively.

The relative stability of this virus in cured tobacco accounts, in part, for the prevalence of mosaic in some fields. Unpublished data collected from field observations in Kentucky over the past 12 years by the junior writers indicate that farms on which workmen use viruliferous non-manufactured tobacco while handling young plants have, on an average, a much higher percentage of field mosaic early in the season than those on which workmen

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Now with United States Department of Agriculture, Bureau of Plant Industry, Washington, D. C.

either do not use tobacco or chew or smoke manufactured tobacco. It is apparent that infectious tobacco, even though several years old, prepared into twists for chewing and smoking, is a source of mosaic if workmen handle it while weeding, pulling, or otherwise handling succulent plants. The incidence of mosaic in fields might, therefore, be appreciably reduced if workmen used tobacco that had been rendered noninfectious. Steam sterilization of twist tobacco has given satisfactory results in mosaic control, but the flavor is changed sufficiently to be objectionable to some men, and steam sterilization is not always accessible to the tobacco grower. Twist tobacco containing about 10 per cent of moisture can be sterilized by heat in an ordinary baking oven. The tobacco is changed somewhat in flavor and contains a larger amount of readily water soluble material and is, therefore, stronger, but men who have used it have not objected.

MATERIALS AND METHODS

Infectious materials used in these experiments were 1936 field-grown, barn-cured White Burley tobacco containing, respectively, yellow, green, and burning strains of tobacco-mosaic virus. A part of the tobacco, after being dried 7 days at room temperature over concentrated sulfuric acid, was powdered, portioned into glass vials plugged with cotton, and exposed to heat in an electric oven. The remaining material was made into twists, which were allowed to dry in the air of the laboratory before exposure to heat. Infectious green tobacco was exposed to some of the temperatures without previous drying.

Virus infectivity of the samples was measured by the local-lesion method with primary leaves of beans of the variety Scotia. One gram of the nontreated and treated material was thoroughly macerated in 10 mls. of 0.1 molar solution of disodium phosphate (reaction of about pH 8.5) and allowed to stand an hour at room temperature. The extract from the macerated tissues was applied by means of an extract-saturated gauze bandage to the upper surface of 10 leaves of the test plants, which were then rinsed with running tap water to remove extraneous materials. Local lesions were counted 6 days after inoculation.

Total moisture content of the samples was taken as the loss in weight after heating for 7 days at 105° C. From these figures the moisture content of the samples was computed from their loss in weight during the treatments.

RESULTS

Data on the moisture content (1 determination) and virus infectivity (average of 3 tests) of the various samples are recorded in table 1. They indicate a similarity for tolerance to dry heat among the 3 strains of virus in cured tobacco. At 70° C. all the samples remained infectious after the maximal exposure of 60 hours, although there was a reduction of infectivity with duration of exposure. At 80, 90, 100, 110, 120, 130, 140, and 150 degrees C. complete inactivation times were 50, 20, 10, 5, 1, 0.5–0.4, 0.4 and 0.4

TABLE 1.—*Moisture content and virus infectivity of cured tobacco after heating at various temperatures for different periods of time*

Exposure		Moisture content[a]				Virus infectivity[b]			
Temp. (°C.)	Time (hrs.)	Yellow mosaic	Green mosaic	Burning mosaic	1882 mosaic	Yellow mosaic	Green mosaic	Burning mosaic	1882 mosaic
70	0	10.1	13.5	9.8	7.9	120.8	143.2	193.2	2.5
"	10	5.7	9.4	6.8	5.2	58.1	31.2	41.8	1.8
"	20	5.7	9.3	6.2	5.1	22.1	7.4	20.1	2.1
"	40	5.6	8.4	5.9	3.9	3.6	2.5	6.3	1.8
"	60	4.8	8.1	5.0	3.4	3.9	3.4	6.3	1.2
80	0	10.1	13.5	9.8	7.9	125.5	113.0	147.4	10.6
"	10	5.9	7.9	5.5	5.0	14.7	21.6	14.1	8.5
"	20	5.3	8.2	6.1	4.4	32.3	6.3	13.6	1.4
"	30	4.6	7.5	5.4	3.2	4.4	1.2	3.2	0.6
"	40	4.7	8.0	4.8	3.0	0.08	0.1	0.1	0.1
"	50	4.3	7.7	4.2	3.1	0.00	0.0	0.0	0.0
90	0	10.1	13.5	9.8	7.9	106.3	126.4	182.5	14.2
"	2	6.2	8.7	7.9	6.0	37.5	14.5	71.3	9.2
"	5	6.3	8.0	6.5	5.1	28.4	28.4	4.3	7.3
"	10	5.7	7.4	4.6	4.4	2.7	1.0	2.3	1.6
"	20	5.1	7.5	4.2	3.8	0.0	0.0	0.0	0.0
100	0	10.1	13.5	9.8	7.9	131.9	98.0	185.8	19.3
"	2	5.9	8.7	5.9	5.1	13.6	14.1	16.5	6.2
"	5	5.7	6.4	4.4	5.0	8.3	4.2	7.1	2.7
"	10	5.2	6.0	4.4	4.8	0.0	0.0	0.0	0.0
110	0	10.1	13.5	9.8	7.9	115.3	123.6	148.6	14.5
"	1	7.0	9.0	6.9	5.1	40.2	40.5	16.3	15.3
"	2	5.8	8.0	5.7	5.0	1.9	3.8	11.2	10.6
"	5	5.5	6.3	5.2	4.7	0.0	0.0	0.0	0.0
120	0.0	10.1	13.5	9.8	7.9	105.7	142.4	160.8	8.9
"	0.3	9.3	10.5	7.8	6.8	55.2	73.4	62.5	2.6
"	0.5	8.1	9.8	7.1	6.0	1.5	6.8	8.6	1.6
"	0.7	7.5	9.2	6.9	5.8	1.4	0.08	2.1	0.5
"	1.0	6.8	8.7	6.1	5.0	0.0	0.00	0.0	0.0
130	0.0	10.1	13.5	9.8		123.4	112.3	145.9	
"	0.2	8.6	11.0	7.9		70.2	23.8	28.8	
"	0.3	7.7	10.3	7.8		11.2	3.1	9.2	
"	0.4	7.4	9.4	7.5		1.0	0.0	0.3	
"	0.5	7.2	9.2	6.9		0.0	0.0	0.0	
140	0.0	10.1	13.5	9.8		137.4	103.9	118.3	
"	0.1	8.1	10.4	7.4		62.0	65.1	86.6	
"	0.2	7.9	8.7	7.2		7.1	14.3	19.8	
"	0.3	7.0	8.6	6.7		0.7	1.0	0.3	
"	0.4	6.9	8.1	6.8		0.0	0.0	0.0	
150	0.0	10.1	13.5	9.8		127.4	149.1	133.7	
"	0.1	7.9	9.4	7.0		88.8	86.6	74.0	
"	0.2	7.4	8.3	6.9		9.0	19.5	23.6	
"	0.3	6.8	7.9	6.5		0.07	0.3	0.1	
"	0.4	6.3	7.7	6.3		0.00	0.0	0.0	
150[c]	0.0					118.5	139.7	124.3	
"	0.1					42.3	85.1	64.3	
"	0.2					12.1	6.6	39.2	
"	0.3					8.5	1.2	2.0	
"	0.4					0.3	0.04	0.01	
"	0.5					0.0	0.00	0.00	

[a] Per cent moisture content based upon weight at 105° C. for 7 days.
[b] Average number of lesions per leaf from 3 tests on 10 leaves each.
[c] Tobacco made into twists for exposures.

hours, respectively. Partial inactivation was proportional to the length of exposure at the various temperatures.

Virus in green tobacco (92 per cent moisture) remained infectious after the maximal exposure of 60 hours at 70° and 80° C. At 90°, 100°, and 110° C. it was completely inactivated in 2, 2, and 1 hours, respectively. The presence of considerable moisture in the green tissues, no doubt, influenced the rate of inactivation of virus in this material. Green tobacco was not exposed to the other temperatures, because the plants in the field were killed by frost.

The moisture content and virus infectivity diminished with the increase of exposure time and temperature. However, a critical point of desiccation associated with inactivation of virus is not apparent from these data.

DISCUSSION

The three strains of tobacco-mosaic virus in cured tobacco possessed similar tolerance to heat. The rate of destruction and complete inactivation was in accordance with the expected relationship—increase in temperature or in time of exposure, with the other factor constant, decreased the infectivity. Complete inactivation of virus in the samples at various temperatures is shown in figure 1. At the higher temperatures, where the time

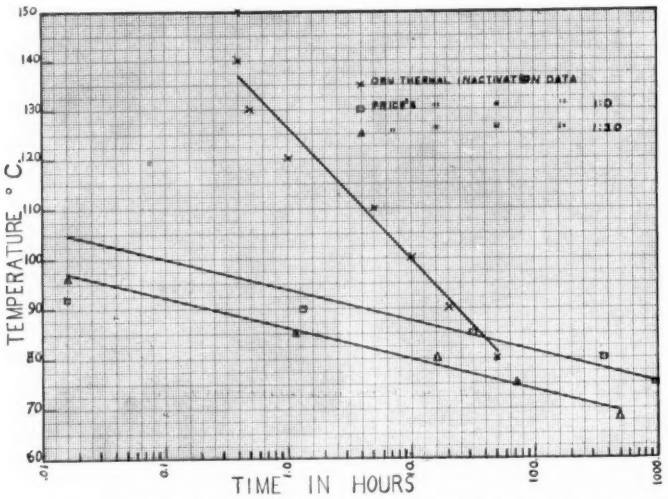

FIG. 1. Data on thermal inactivation of tobacco-mosaic virus in cured tobacco and that of virus in aqueous solutions by Price (5) plotted as functions of temperature and time.

necessary for inactivation is short, the lag in inactivation is similar to that ascribed to time of penetration of heat by Bigelow (2) with thermal-death-time curves for bacteria. At the lower temperatures the time for penetration of heat has no appreciable effect because of its small proportion to the total period of exposure. Thermal inactivation of tobacco-mosaic virus in solutions (5) when plotted on the same scale (Fig. 1) shows a different rate of destruction according to the relative slope of the curves. These variations

suggest different mechanisms of inactivation the nature of which is not apparent. However, the time for inactivation of virus in solution and that in dry tissue is approximately equal at the lower temperatures. The greater effectiveness of moist heat upon inactivation is expected, since living vegetative and resting cells or native proteins are more rapidly destroyed by heat when they are in solution than when dry. Spores of *Bacillus subtilis*, previously dried (6), required 2 hours of heat at 140° C. for destruction, while proteins (egg albumen and antigens in pollen), dried and exposed to the same temperature, retained their specific antigenicity or their native configuration. According to theories, heat does not denaturize proteins when in a dry condition. Intolerance of the crystalline-protein virus to drying at room temperature (7), destruction of virus activity by the action of certain chemicals that do not change the antigenic specificity (9), and the high molecular weight of the active protein molecule (8) indicate a unique relationship essential to the biological property of infectiousness and to regeneration within the susceptible host tissue. Since purification of the virus increases its susceptibility to inactivation upon drying, virus in expressed plant juice, or in cured or dried tissues, appears to be protected by substances or conditions present in these materials.

Inactivation of virus in the 1882-cured tobacco appears to be similar to that in tobacco recently cured. The differences in virus concentration in the nontreated tissues of the 2 tobaccos do not indicate a diminution of virus upon storage, since the virus concentration of the 1882 sample was not determined previously.

Inactivation of virus in green tissue containing 92 per cent moisture at the beginning of the experiments was, in general, intermediate between virus in solution (5) and that in dried tissues. Evaporation of water from the surface of the tissues and presence of water vapor in them probably accounted for this relationship. Since the tissues exposed at 70° and 80° C. for 60 hours lost 8.5. and 87.3 per cent of their moisture, respectively, evaporation of water from the surface may have been sufficient to prevent the heating to the point of virus inactivation, considering 80° C. for 50 hours sufficient for inactivation of virus in dried tissue. At 90°, 100°, and 110° C. the time for inactivation of virus in green tissues was less than that in dried tissues. In this case evaporation, no doubt, reduced the temperature of the tissues, but the internal areas were heated to the point of virus inactivation under the pressure of water vapor arising from water in the tissues at these temperatures. Protozoa show greater susceptibility (3) to heat destruction in moist soil (50°–60° C. for 10 minutes) than in dry soil (80°–85° C.).

Since these data indicate that tobacco-mosaic virus in cured tobacco, either in leaf or in twist form, may be inactivated (150° C. or 302° F. for 0.5 hour) within the limits of an accessible kitchen range, tobacco, to be used for chewing or smoking by workmen, may be rendered noninfectious by such a treatment at home. Since newspaper browns at this temperature, it might

be used as an indicator for the temperature to destroy virus in the absence of suitable thermometers. Importance of the destruction of virus in cured tobacco is apparent when viruliferous tobacco used by workmen appears to be the principal source of inoculum for tobacco beds and fields under conditions in Kentucky.

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HOST RANGE AND IDENTITY OF THE SMUT CAUSING ROOT GALLS IN THE GENUS BRASSICA

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INTRODUCTION

A smut causing root-galls in mustard, *Brassica campestris* L. var. *sarson* Prain, was first recorded in India by Mitra (6). Its pathogenicity was proved in pot experiments, and the smut was identified at the Imperial Mycological Institute, Kew, as *Urocystis coralloides* Rostrup. It was then supposed that the disease was confined to a single field in a village near Pusa, but affected plants have since then been collected in other villages and the disease does not seem to be so local as was once presumed. Attempts to confirm the identification and determine the host range of the smut have been made again, and the results are herewith reported.

SYMPTOMS OF THE DISEASE

In the field, affected plants are distinguishable by their stunted stature in the flowering stage. The leaves are pale and the plants apparently flower

[1] Grateful acknowledgment is made to Mr. S. F. Ashby, Director, Imperial Mycological Institute, Kew, for kindly obtaining on loan a part of Rostrup's type specimen, for going through the typescript, and for suggestions. Thanks are due to Mr. M. Azmutullah Khan of this section for the help he gave in washing the roots and in measuring the spores.

earlier than healthy ones. If pod formation has started, the pods are empty or contain very few seeds, which may be sterile. No other symptoms are evident on the parts aboveground and it is difficult to identify the disease with certainty without previous experience. If such plants are dug up by gently loosening the soil so as not to break the finer roots, large lumps of earth will be found clinging to the roots; if this soil is carefully washed out, the galls can be distinguished. If the galls are mature, they may have commenced to disintegrate; immature galls will, however, adhere to the roots. They are more numerous on the finer roots but break off easily because of their weight.

FIG. 1. A. Sarson (*Brassica campestris*) plants showing root galls. B. Sarson plants growing in non-infested (pot at upper left) and smut-infested soil.

The galls are of various sizes, some as large as a pea and others measure 1½ inches in diameter. (Fig. 1, A and 2). They are irregular in outline, wart-like, cream-color when immature and lead grey, because of the presence of dark brown spores within, when mature. The surface of the galls is smooth and slightly glistening.

It is possible that mustard-crop failures attended by stunted growth of plants and reduced yields, usually ascribed to improper fertilization or other soil and environmental factors, may be due also to attack by this smut, a possibility that should not be lost sight of. An examination of the underground parts of the plants should, in such cases, invariably be made to discover if any smut galls are present.

As the disease has been seen in only a few fields, and is not easily distinguishable unless the plants are pulled out, which it is not possible to do

indiscriminately in the farmers' fields, no accurate data on losses are available. The size and number of the galls make it clear, however, that much of the food material elaborated for the production of a luxuriant growth and good yield, is used up in their formation and losses might, therefore, be appreciable.

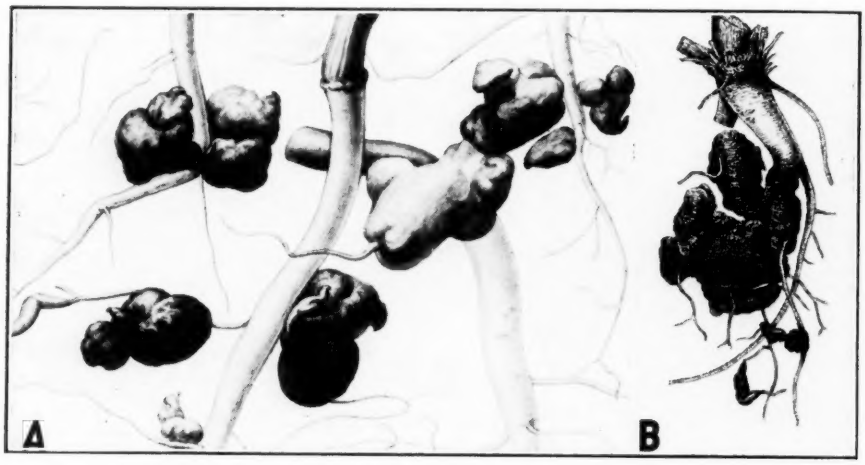

FIG. 2. A. Galls on roots, drawn from nature. ×3. B. Galls formed on *Turritis glabra* by *Urocystis coralloides*. Reproduced, with permission, from J. Lind's Danish Fungi.

POT EXPERIMENTS TO DETERMINE LOSS

In order to determine the extent of loss incurred, pot experiments were made. Soil for these experiments was obtained from a field where considerable smut was present, and a field where no such smut was found at any time. Further, in an infested field, several plants were uprooted, after harvest, and the galls collected for artificial infestation of the soil. The soil was potted as shown in table 1.

TABLE 1.—*Effect of smut infection on height and yield of mustard*

	Nature of soil	No. of pots	Total No. of plants	Mean height of plants	Average yield
				cm.	*g.*
I	Naturally infested soil	10	50	58.8	0.488
II	Same as I, but reinfested by smut	10	50	60.3	0.242
III	Noninfested soil	10	58	106.7	0.828
IV	Same as III, but artificially infested by smut	10	78	100.4	0.594

All the pots were uniformly fertilized with barnyard manure and kept moist by frequent watering throughout the summer of 1935. On October 25, 5 to 8 seeds of sarson were sown in each pot. Germination was satisfactory and the plants made normal growth until about November 10. From

that time onward, growth of plants in some of the pots lagged and the heights of the different plants recorded a month later showed this to be true. The results are recorded in column 4, table 1.

When the plants began to bloom, it was noted that those in infested soil flowered about 5 days earlier than those in smut-free soil. Seed formation in those plants also was indifferent. The yields per plant are recorded in the last column of table 1.

The plants were then uprooted to observe gall formation. No galls had formed in those growing in pots of series III, which contained noninfested soil, but galls were present in those growing in infested soil.

ANALYSIS OF THE RESULTS

The data recorded in table 1 indicate that the plants in naturally infested soil make poor growth, their height being especially affected. On the addition of crushed galls to soil not naturally infested, the height of the plants is lowered, presumably because of fungus infection. The data further indicate a striking decline in yield because of infection. The yield of those plants growing in naturally infested and reinfested soil was reduced by about $\frac{1}{4}$ normal, while in naturally infested soil, alone, the yield was reduced almost 50 per cent. In artificially infested disease-free soil, there was an appreciable decline.

It is further manifest from these data that galls are a factor in the dissemination of the smut. Mitra (6) was unable to induce gall formation when plants were grown in smut-free soil that had subsequently been artificially inoculated. Presumably the quantity of the inoculum used determines whether smut infection would or would not take place.

These results clearly show what a menace this smut can be to the mustard crop and point the necessity of a correct diagnosis of mustard-crop failures to determine the extent to which this smut may be responsible.

HOST RANGE OF THE SMUT

The determination of the host range of the smut among the other brassicas, many of which are useful as a source of oil seed or as vegetables, was made in 1936. The soil saved from the preceding year's pots was used for this purpose, and the following hosts were available:

Sarson (Indian mustard)	Oil seed	*Brassica campestris* L.
Black mustard	Oil seed	*Brassica nigra* Koch
Rai	Oil seed	*Brassica juncea* Coss
Toria	Oil seed	*Brassica napus* L.
Turnip	Vegetable	*Brassica rapa* var. *lorifolia* Bailey
Radish	Vegetable	*Raphanus sativus* L.
Cabbage	Vegetable	*Brassica oleracea* var. *capitata* L.

Three pots were provided for each host and in each pot about 10 plants were raised. The plants matured and were ready for examination about the end of January, when the roots were carefully washed and the galls collected. All hosts used in these tests were attacked, but the largest number

of galls was formed on sarson and turnip and the least on cabbage and toria. The galls on cabbage and toria were about 3 mm. in diameter but those on sarson ranged from 8 to 22 mm.

All these hosts are therefore liable to be attacked, though the number and size of the galls formed may vary. It is further apparent that on congenial hosts the smut forms large and numerous galls, but that on less congenial hosts, gall production is very much reduced.

FORMATION OF GALLS

In order to determine just when gall formation began, pot cultures of sarson plants growing in smut-infested soil were dug up every week. The soil was loosened by a forcible jet of water and the rootlets were carefully collected for examination. The first galls were observed 3 weeks after sowing, in the form of irregular, flattened root swellings. They were cream white, and, on sectioning, showed the intercellular mycelium; spore formation was in its incipiency and spore balls became evident in the fourth week. Thereafter, their number gradually increased as the size of the galls increased.

The washed galls were placed for a few weeks in lacto-phenol, which served both as a killing and a fixing fluid, and then embedded in paraffin and sectioned from 5 to 10 μ thick.

In galls 3 weeks old the mycelium was confined to the cortical parenchyma where it was strictly intercellular; no haustoria were observed, but the nutritive mycelium was more prominent than the sporogenous mycelium. The host parenchyma looked as though it had been torn asunder by the invading hyphae, and large intercellular cavities full of mycelial filaments were evident here and there in the cortex. Dissolution of the middle lamella and the cell walls of the parenchyma led to an increase in size of these cavities. Sections of galls 4 weeks old showed abundant spore-ball formation. (Fig. 3). In the more advanced galls the mycelium had invaded the stelar parenchyma.

The sporogenous mycelium stained a little deeper than the nutritive, and

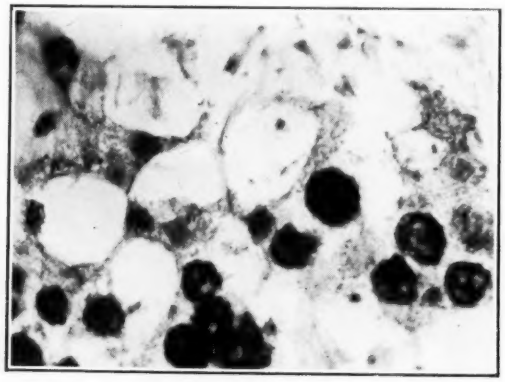

FIG. 3. Photomicrograph of a section of a young gall. Note intercellular nutritive mycelium and spore balls.

the filaments were apparently disposed in whorls. These whorls were the spore-ball initials, and in the centre of each there were 1 to 5 prominent, thick-wall cells, the spores. The rest of the mycelium in the immediate vicinity comprised the sterile layer of cells; and all of them, the central fertile cells and the outer sterile ones, were held together firmly, forming a pseudoparenchymatous structure, the spore-ball.

As the galls increased in size and age, much of the parenchymatous tissue was replaced by the spore balls, but the stele retained its individuality, assuming a tortuously curved position within the gall. There was no abnormal increase in size of the parenchyma cells nor in their number, but there was some increase in the size of the xylem elements. The mature galls were made up of the spore balls, a hyaline fungal tissue composed of anastomosing and agglutinated mycelial strands, and the xylem tissues.

IDENTITY OF THE SMUT

In the earlier paper (6) this smut was referred to as *Urocystis coralloides,* but the appearance of the galls and the size of the spore balls suggested that it might be different. With a view to determining the validity of the earlier identification, the following points were investigated: 1. Comparison of the galls formed on *Turritis glabra* L., in Denmark and those formed in India on Indian mustard. 2. Infection experiments to determine whether the Indian smut is pathogenic to *T. glabra.* 3. Comparative study of the spores of the type material of *U. coralloides* and those of the Indian smut.

Urocystis coralloides was described by Rostrup (7) as the cause of root galls on the cruciferous plant *Turritus glabra,* in Denmark. Efforts to obtain specimens of the gall-bearing plants were unsuccessful, but, in reply to an enquiry, J. Lind, the Danish mycologist, kindly sent a copy of the illustration of the galls in his book, *Danish Fungi.* It will be noted from this (Fig. 2, B) illustration that the galls on *T. glabra* are cylindrical, unilaterally diffused enlargements, some of which are of coral-like shape. The galls formed on Indian mustard do not look like corals but are more wart-like, tuberculate, wrinkled, and globular.

Pathogenicity tests to determine whether the Indian mustard smut was or was not parasitic on *T. glabra* were rendered possible due to the kindness of the Director, Universitates Botaniska Museum of Copenhagen, Denmark, who sent seeds of that plant. Lagerheim (4) found a root-gall smut at Montpelier on *Matthiola sinuata* L., another cruciferous plant, and identified it as *Urocystis coralloides.* It is not clear from Lagerheim's account whether he had compared his smut with the type material. In these pathogenicity tests, however, plants of *M. sinuata* also were included, seeds of which were kindly supplied by the Director, Royal Botanic Gardens, Kew.

None of the seeds sown at Delhi germinated, but at Pusa 4 seeds of *Matthiola sinuata* and 7 of *Turritis glabra* germinated. The seedlings were transplanted in October, 1936, to pots containing smut-infested soil, one pot being sown to sarson, as a control. The plants were uprooted in February,

when quite mature. Galls were present in the sarson plants, but there were no galls nor even swellings seen in those of *T. glabra* and *M. sinuata*. These experiments indicated that the Indian smut was not pathogenic to either of these hosts.

A comparative study of the spores of Indian mustard smut and those of the type of *Urocystis coralloides* was rendered possible through the kindness of Mr. S. F. Ashby, Director, Imperial Mycological Institute, Kew, who obtained, on loan, a small piece of Rostrup's type specimen from the Botaniska Museum, Copenhagen. Preparations were made in lacto-phenol and measurements of 50 spore balls and over a hundred fertile and sterile spores of each specimen were made. The results are recorded in table 2.

TABLE 2.—*Linear measurements of the spore balls of the mustard smut and of Urocystis coralloides*

Materials	Indian mustard smut		*Urocystis coralloides*	
	Length	Breadth	Length	Breadth
	μ	μ	μ	μ
Sport balls				
Range	25–58	20–45	25–44	18–40
Mean	37.7	32.2	33.6	27.9
Fertile spores				
Range	13–25	10–20	14–24	10–20
Mean	20.3	15.7	19.2	15.1
Sterile spores				
Range	5–15	3–10	3–12	3–10
Mean	9.9	6.1	9.2	5.4

It will be noted from the data recorded in table 2 that the Indian smut has larger spore balls and larger fertile and sterile spores. The difference in mean values in each instance also is statistically significant.

The number of fertile spores in a spore ball has a diagnostic value in the genus, but instead of the means, the frequencies are given below, from which it will be seen that the Indian smut has a larger number of fertile spores per spore ball than has the Danish smut.

Number of cells	Indian smut; frequency	Danish smut; frequency
1	18	24
2	20	17
3	7	6
4	3	2
5	2	1

The comparative study made by the writer shows that in the shape of the galls, the nonpathogenicity of the Indian smut to the hosts to which *Urocystis coralloides* is parasitic, and in the size of the spores, the Indian smut differs from the Danish smut. It is, therefore, considered as a new species and the name *Urocystis brassicae* is proposed for it.

Urocystis brassicae n. sp. Spore powder, black, compact; spore balls 38 × 32 μ, range 25–58 × 20–45 μ with 1–5 fertile cells, 2 or 3 predominating. Fertile spores deep brown,

20×16 μ, range $13–25 \times 9–20$ μ, surrounded by numerous small, bright brown, somewhat longish, sterile spores forming a continuous layer. Spores formed in wart-like, tuberculate, irregular, lead-grey and smooth galls. Habitat: Underground parts of *Brassica campestris* L. and other *Brassica* spp.

Urocystis brassicae, species nova. Sporis atram massam pulverulentam efformantibus, presse compactis; glomerulis sporarum 38×32 μ, vel inter $25–58 \times 20–45$ μ variantibus, cellulis, fertilibus una usque ad quinque, saepius duobus vel tribus instructis. Sporis fertilibus atro-brunneis, 20×16 μ vel inter $13–25 \times 9–20$ μ variantibus, multis et parvis sterilibus sporis flavo-brunneis, longiusculis circumtectis continuum stratum efformantibus. Sporis in verruculosas et tuberculatas gallas irregulares plumbeo-glaucas leves congregatis.

Hab. in partibus subterraneis *Brassicae campestris* et aliarum specierum Brassicae.

Type specimens deposited in the Herbarium of the Imperial Agricultural Research Institute, New Delhi, Imperial Mycological Institute, Kew, and the New York Botanic Garden.

A smut, characterized by galls on the roots of yet another cruciferous plant, *Sophia andrenarum* Cockerell, was discovered in 1903 by Griffiths (3) in the United States and called by him *Urocystis sophiae.* This smut also forms wart-like tubercles, but the galls are smaller than those of the Indian smut. The spore balls of *U. sophiae* have a diameter range of 30–45 μ and the fertile cells are 15 μ in diameter. The size of the galls and that of the spores are thus much smaller than those of the Indian smut. Part of Griffiths' type specimen, secured by S. F. Ashby from G. M. Reed of the Brooklyn Botanic Garden, was lost in the mail, and no comparative study, therefore, could be made. Seed of *S. andrenarum* obtained from the United States did not germinate.

Rabenhorst founded the genus *Urocystis* to separate it from the older Friesian genus *Tuburcinia,* because the former had a continuous layer of sterile spores, apparently absent in the latter. Liro (5) contends, however, that such a layer is present in both genera and, in consequence, has merged the genus *Urocystis* into the older genus *Tuburcinia.* Germination studies by Brefeld (1, p. 180) of the spore balls of *T. primulicola* Bref., and by Woronin (8) of *T. trientalis* Berk. et Broome had shown that the peripheral cells in this genus are not sterile. Dietel (2), therefore, has retained the genus *Urocystis* and has been followed in the present paper.

SUMMARY

Symptoms of a smut that produces root galls in *Brassica campestris* are described and it is experimentally shown that considerable reduction in yields may result from attack by this fungus. The smut is soil-borne and the galls are capable of spreading the disease from place to place.

Besides Indian mustard, several other species of *Brassica,* including radish, turnip, and cabbage, are attacked by it.

The smut is not capable of infecting *Turritis glabra* nor *Matthiola sinuata,* species to which *Urocystis coralloides* is pathogenic. This fact and the spore measurements indicate that the Indian smut is not *U. coralloides* and that it is a new species. The name *Urocystis brassicae* is, therefore, proposed for it.

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THE ASSOCIATION OF BUNT WITH LOOSE SMUT AND ERGOT[1]

W. F. HANNA

(Accepted for publication Oct. 20, 1937)

The occurrence of two or more parasitic fungi on the same plant is a matter of common observation. For example, wheat plants attacked by smut may also be infected by one or more of the rusts, by mildew, and by leaf-spotting fungi. Concurrent invasion of the ovaries of plants by two parasitic fungi, however, is a phenomenon met with much less frequently. Two instances of this double infection of the ovaries of wheat observed by the writer were recorded in a brief published abstract (2). The fungi associated in the infected ovaries were those causing bunt and loose smut, and bunt and ergot. The first of these associations has been noted by other workers, but the second, so far as the writer is aware, was recorded for the first time. In the present paper it is proposed to describe briefly these double infections, and to present photographs of portions of the diseased plants.

BUNT AND LOOSE SMUT OF WHEAT

Spores of loose smut, which gain entrance to the wheat floret, germinate on the stigma about the time of anthesis and invade the young ovary. The mycelium of the parasite becomes localized in the embryo and, at the time of seed germination, passes into the growing point. Later, as the plant develops, the inflorescence is invaded and converted into a mass of spores. Not infrequently spores are produced also in the leaves and, according to Klushnikova (3), the presence of the fungus has been established in the young roots. Infection by *Ustilago tritici* (Pers.) Rostr., therefore, may be regarded as a highly systemic invasion.

Infection by the bunt fungi, *Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kühn, occurs soon after the wheat seed has commenced to germinate and while the young plumule is still only a few millimeters in length. As the

[1] Contribution No. 514 from the Division of Botany, Experimental Farms Branch, Dominion Department of Agriculture, Ottawa, Canada.

host plant approaches maturity its ovaries are invaded by the parasite and converted into bunt balls. The anthers of infected flowers fail to dehisce, and apparently it is the unfertilized ovary that is stimulated to develop into a bunt ball. It has occasionally been noted (1) that bunt spores are produced in the leaves as well as in the ovaries, an observation that suggests complete invasion of the infected plant. Infection of a single wheat plant by the two species of bunt may sometimes occur. Smith (6), who inoculated wheat seedlings with a mixture of *T. tritici* and *T. laevis* spores, found that approximately 50 per cent of the bunted plants were infected by both species. In those plants in which both species were present it was usually found that individual heads bore the spores of one species only, but in a few instances spores of both species were found in a single bunt ball.

Both Munerati (5) and Milan (4) have subjected loose-smut-infected seed to inoculation with bunt spores (*Tilletia tritici*). The percentage of bunt infection in plants grown from this seed was invariably less than in plants grown from seed inoculated with bunt alone. A small percentage of the seeds inoculated with the two smuts produced plants bearing both bunt and loose-smut spores in the same head.

In the summer of 1931, at Winnipeg, a few heads of Kota wheat growing in the smut plots were observed to be infected by both loose smut and bunt (*Tilletia tritici* or *T. laevis*). Usually the lower part of the spike was destroyed by loose smut, whereas the upper portion contained a few bunt balls. The seeds from which these plants grew had been artificially inoculated with spores of *T. tritici* or *T. laevis*, but the loose smut was the result of natural infection.

A few months before these observations were made, seed of Kota wheat, artificially inoculated with loose smut, was exposed to infection by bunt (*Tilletia tritici*) and grown to maturity in pots in the greenhouse. In the same experiment there were included the following lots of seed of this variety: (1) Noninoculated seed; (2) seed inoculated with *Ustilaga tritici* alone; (3) seed inoculated with *T. tritici* alone.

In the experiment just referred to, the bunt inoculations were performed by infecting young seedlings with the primary conidia (basidiospores) of *Tilletia tritici*. The lots of seed were germinated on moist sand in Petri dishes. When germination had commenced, another Petri dish containing non-nutrient agar on which bunt spores were germinating was inverted above each dish of young seedlings. This permitted the primary conidia discharged from the germinating spores to shower down upon the seedlings in the dish below. During the period of inoculation (7 days) the dishes were kept in a chamber maintained at 12°–15° C. After inoculation the seedlings were planted in the greenhouse in pots of sterilized soil. The noninoculated seed and that inoculated with *Ustilago tritici* alone were likewise germinated in moist sand and transferred to pots of sterile soil. Records were kept of the number of seeds placed to germinate, the number of plants produced, and particulars of the smut infection. The results are summarized in table 1.

TABLE 1.—*Inoculation of Kota wheat with loose smut (Ustilago tritici) and bunt (Tilletia tritici)*

Inoculation	Seeds inoculated	Plants produced	Survival	Smutted plants	Smutted plants
	No.	*No.*	*Per cent*	*No.*	*Per cent*
Noninoculated	100	97	97	0	0
T. tritici	112	81	72	70	86
U. tritici	100	74	74	49	66
U. tritici and *T. tritici*	100	44	44	41	93

It is a matter of some interest that the heavy smut infections, whether by *Ustilago tritici*, *Tilletia tritici*, or by both species, were associated with high seedling mortality. This is particularly noticeable in the lot of seedlings infected with both smuts, where only 44 per cent of the seed produced plants that survived to maturity. Results such as these show that the harmful effect of smut infection is not confined to destruction of the inflorescence, but may manifest itself also in injury to seedlings, with a consequent reduction in stand. The importance of this type of injury has been emphasized by Zade (7, 8), who suggested that the favorable effect of seed treatment on the yield of smut-resistant varieties of cereals might be due to the control of latent infection.

Table 1 shows that 41 of the 44 plants inoculated with the two species of smut became infected. The distribution of infection according to species is given in table 2. A majority of the plants showed infection with only one species of smut. The percentage infected with *Ustilago tritici* alone was about equal to that obtained from inoculation with this species only, indicating that the loose smut had not suffered from competition with bunt. The suppression of bunt in the presence of loose-smut infection was quite pronounced, as may be seen from a comparison of the precentages of plants infected with *Tilletia tritici* in tables 1 and 2. The 3 plants which produced spores of both smuts had a total of 7 heads, 4 of which were invaded by *U. tritici* and *T. tritici*, whereas the remaining 3 bore spores of *U. tritici* alone. In all of the heads infected by the two smuts the lower portion was occupied by *U. tritici* and the upper portion by *T. tritici*. The appearance of heads infected with the two smuts is shown in figure 1, A.

TABLE 2.—*Distribution of infection in plants of Kota wheat inoculated with Ustilago tritici and Tilletia tritici*

Species	Smutted plants	Smutted plants
	No.	*Per cent*
U. tritici	28	63.6
T. tritici	10	22.7
U. tritici and *T. tritici*	3	6.8
Noninfected	3	6.8

BUNT AND ERGOT

In the summers of 1931 and 1932, sclerotia of ergot, *Claviceps purpurea* (Fr.) Tul., were observed in bunted heads of wheat plants growing in the smut plots. On closer examination it was found that bunt balls containing spores of *Tilletia tritici* were fused to the projecting tips of many of the sclerotia. Composite structures of this kind were found on the following varieties of wheat: Little Club, Reward, Mindum, and Reward × Garnet (Fig. 1, B and C). The manner in which these bodies originate has not been

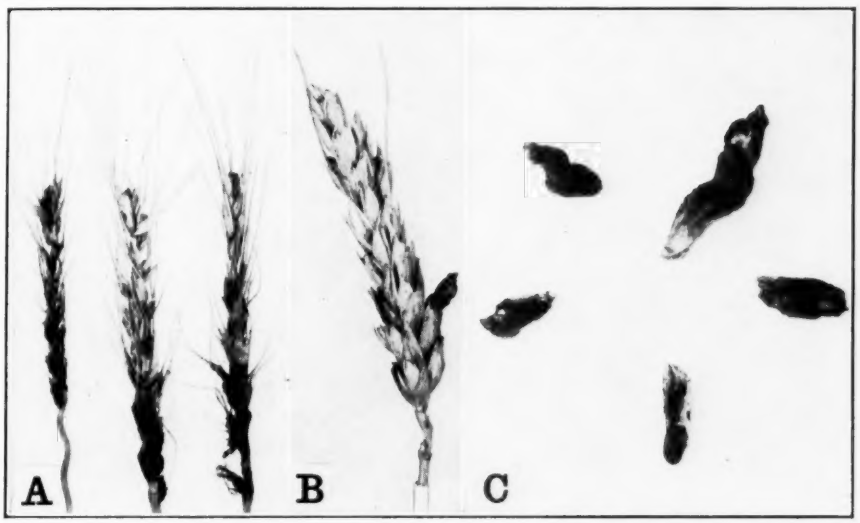

FIG. 1. A. Heads of Kota wheat infected with *Tilletia tritici* and *Ustilago tritici*. The upper portion of each head contains bunt balls. B. A bunted head of Mindum wheat. A bunt ball, fused to the tip of an ergot sclerotium, projects from one of the florets. C. Diseased wheat grains, each one composed of an ergot sclerotium and a bunt ball. The outer portion of each grain is filled with spores of *Tilletia tritici*.

investigated, but the regular occurrence of the bunt spores at the outer tip of the ergot body suggests that they may develop from partially bunted kernels. When the florets of bunted wheat plants are examined it is found that the ovaries have been invaded at a very early period in their development. It sometimes happens, however, that a part of the ovary escapes infection and, after fertilization, develops into healthy tissue. Grains of this kind in the early stages of development might be subject to infection from ascospores or conidia of the ergot fungus. This secondary infection would convert the healthy tissue into an ergot sclerotium.

SUMMARY

Heads of Kota wheat infected with both *Ustilaga tritici* and *Tilletia tritici* have been collected in the field. Heads of this variety infected by the two smuts were produced also in the greenhouse by artificial inoculation. In the material examined, bunt has been restricted to the upper and loose smut to the lower part of the spike.

The mortality is high among seedlings inoculated with both *Ustilago tritici* and *Tilletia tritici*. In greenhouse experiments only 44 per cent of the seeds inoculated with the two smuts produced mature plants, as compared with 97 per cent for noninoculated seeds. Competition between *U. tritici* and *T. tritici* resulted in a marked suppression in infection by the latter species.

On several varieties of wheat bunt balls containing spores of *Tilletia tritici* have been found fused to the projecting tips of ergot sclerotia.

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SEASONAL VARIATIONS IN SUSCEPTIBILITY OF TOBACCO TO INFECTION WITH TOBACCO-MOSAIC VIRUS

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(Accepted for publication Dec. 8, 1937)

In connection with experimental studies on the effect of mineral nutrition on host susceptibility to infection with tobacco-mosaic virus, the question arose as to whether this susceptibility is influenced also by fluctuations in environmental conditions due to seasonal changes. The object of this paper is to report briefly the results of 129 consecutive weekly tests that were carried out in an endeavor to answer this question.

MATERIALS AND METHODS

Seedlings of Turkish tobacco, *Nicotiana tabacum* L., were cultured in white quartz sand until they had developed 3 or 4 leaves and were about 2 cm. in height. Once each week 126 uniform plants at this stage of growth were transplanted into 4-inch porous clay pots, filled with composted soil, to which peat moss had been added. These pots were then placed in loose peat moss in a side bench in a greenhouse. One week the pots were placed to the left of the middle of the bench, the next week to the right, the position of the plants on the bench thus being alternated throughout the experiment.

On the 3rd day after potting, 100 plants were inoculated by means of a single puncture with a needle, size 00, in the youngest leaf over 0.5 cm. long on each plant. This method was used so that relatively uniform doses of inoculum might be introduced into each plant. The inoculations were made late in the afternoon to minimize any possible harmful effect of unfavorable light or temperature on the freshly inoculated leaf. The extract of green tobacco-mosaic virus (Johnson's tobacco virus 1) was prepared as follows: Undiluted juice from diseased tobacco plants was frozen for 48 hours in test tubes and then thawed. It was carefully remixed and 2-cc. portions placed in small test tubes. These tubes were tightly corked, sealed with paraffin, and stored in a cold room at $-14°$ C. until used. Each week the juice in one tube was thawed, used for the inoculation, and then discarded. In this way the virulence of virus extract was held as uniform as has been found possible. It is probable, therefore, that variations in the number of plants becoming diseased at different seasons were due to variations in host susceptibility rather than to changes in the inoculum.

The following observations and calculations were made each week: (1) incubation period of the disease, (2) number of plants infected, (3) greenhouse temperature, and (4) hours of sunshine. The temperature record was made by means of a "Tycos" recording thermometer placed on the greenhouse bench beside the test plants. The record of the hours of sunshine was obtained from a substation of the Weather Bureau of the United States Department of Agriculture, located at Trenton, N. J., 12 miles from Prince-

ton. Some variations occurred between the number of hours of sunshine in Trenton and in Princeton, but periodic checks indicate these to be quite small.

EXPERIMENTAL

The susceptibility of the young plants, measured by the percentage of plants that showed symptoms of tobacco mosaic, is expressed graphically in figure 1. Each circle on the curve represents the percentage of infection in

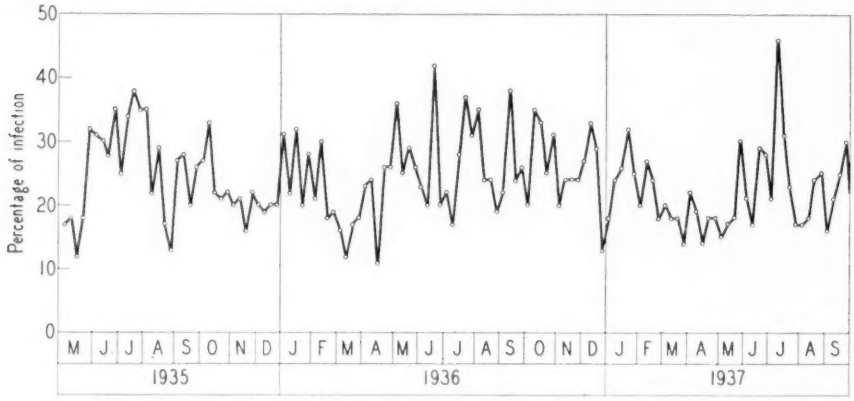

FIG. 1. Susceptibility of young tobacco plants tested at weekly intervals to infection with tobacco-mosaic virus. Each circle represents the percentage of 100 plants which became infected in each test.

a single test. From this graph it is apparent that variations in the number of plants becoming diseased were obtained from week to week. The cause of this variation is difficult to define because of the complex interrelationship of the environmental factors involved. Of these factors, the following are probably the most important: temperature, light intensity and duration, and humidity. A detailed study of these factors was not attempted because of limited equipment, but such observations as were possible with the facilities available were made on each test.

In an effort to illustrate the influence that some of these factors might exert on susceptibility, the graphs in figure 2 have been prepared. Each circle on these 4 curves represents the average of the weekly observations for each 2-month period. The upper graph shows the average hourly temperature in the greenhouse during the day of inoculation and the day following in each test. The second graph shows the average daily hours of sunshine during the same period. The third graph shows the average number of days that elapsed from time of inoculation to the appearance of symptoms of the disease. The lowermost graph shows the average susceptibility of the plants as determined by the percentage of plants that became diseased.

From the graph expressing susceptibility, it is apparent that the plants showed a somewhat definite annual cyclic variation in their susceptibility to infection with tobacco-mosaic virus. In both years the susceptibility was low during late winter and early spring, and high during early summer. In

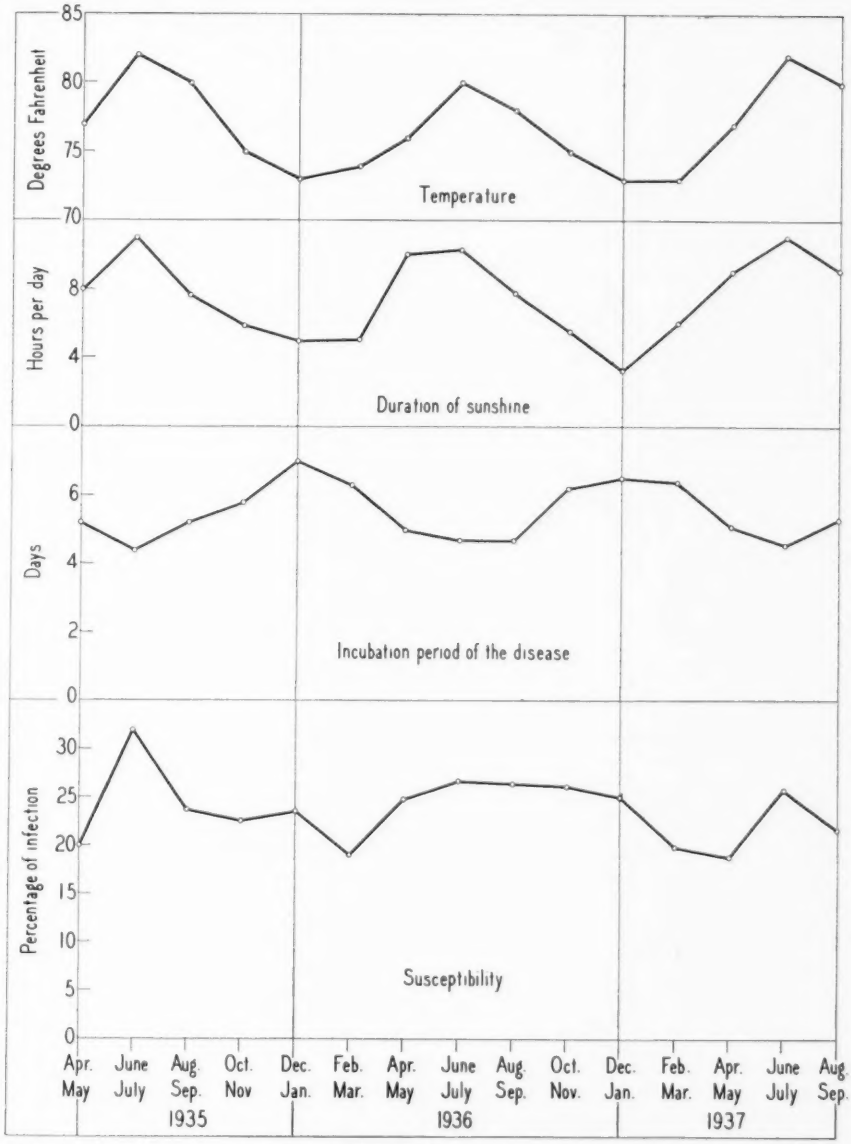

FIG. 2. Graphs showing the relation of temperature and hours of sunshine to incubation period of the disease and the susceptibility of the host. Each circle represents the average of the weekly observations made during each 2-month period.

1935, susceptibility was high during June and July and then decreased about 25 per cent during August and September. The average susceptibility remained fairly constant at this level until February, 1936, when a second decrease was observed. Following an increase in susceptibility in April–May, 1936, no appreciable differences were observed until in February, 1937, when a decrease of about 20 per cent occurred. In 1937 susceptibility decreased gradually from its maximum in June and July.

In an effort to correlate to some extent these variations in susceptibility with seasonal fluctuations in temperature and light, certain salient features in the graphs in figure 2 may be mentioned. The period during which plants were low in susceptibility did not coincide with that in which the average temperature was lowest or the duration of sunshine was shortest, but followed this period by at least 2 months. The lowest average temperature and the shortest duration of sunshine occurred in the December–January period each year, but the plants were lowest in susceptibility during February–March in 1936 and during April–May in 1937. However, in both years the period of high susceptibility coincided with that of high temperature and long duration of sunshine.

The incubation period of the disease showed very marked variations from season to season, and these variations seemed constant from year to year. During June and July the symptoms of the disease appeared about 4 or 5 days after inoculation. During December and January, however, at least 6 or 7 days elapsed before the appearance of the disease. These variations seemed to be definitely correlated with seasonal fluctuations in light and temperature. The incubation period was shortest during June and July, a period characterized by high temperature and long duration of sunshine. Moreover, during December and January when the incubation period was long, the average temperature was lower and the duration of sunshine shorter than at any other time during the year.

The general results of the experiments reported here indicate that the normal variations in host reaction to tobacco-mosaic virus from season to season should be considered in planning certain types of investigations. For example, experiments in which high susceptibility to infection is desirable should be carried out during the summer months. Furthermore, the length of the incubation period of the disease is an important factor in studies pertaining to virus movement. In reporting experiments dealing with the incubation period of the disease, mention should always be made of the seasons in which such experiments were carried out.

SUMMARY

A study involving weekly tests over a period of 2½ years showed definite variations in the susceptibility of small Turkish tobacco plants to infection with tobacco-mosaic virus. Susceptibility was high during early summer, a period characterized by high temperature and long duration of sunshine, and low during late winter and early spring. The incubation period of the disease within the plant showed a direct correlation with seasonal fluctuations in light and temperature, being short during early summer and long during the winter months.

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PHYTOPATHOLOGICAL NOTES

Observations on the Loose Kernel Smut of Johnson Grass.—Although kernel smut has been observed and collected on Johnson grass, *Sorghum halepense* (L.) Pers., in the southern part of the United States for many years, there seems to be considerable confusion concerning the identity of the causal organism. In some cases it apparently has been mistaken for *Sphacelotheca sorghi* (Link) Clinton, and has been mentioned in literature under that name. Specimens of Johnson grass smut collected in Kansas in 1935 and in Oklahoma in 1936 were studied at the Kansas Agricultural Experiment Station, both in the laboratory and in the field. Samples were sent to George L. Zundel, who stated that the organism most closely resembled *S. holci* Jackson as described by H. S. Jackson[1] in 1934. The material described by Jackson was collected by Chardon, Toro, and Alamo on cultivated sorghum in Venezuela.

After the Kansas and Oklahoma specimens had been examined by Zundel and pronounced *Sphacelotheca holci*, the authors called attention to the occurrence of the fungus and stated that the report before the Kansas Academy of Science[2] was the first record of that organism on Johnson grass in North America. In a recent paper, Rodenhiser[3] calls attention to certain characteristics of a smut on Johnson grass, which he describes as a new physiologic race of the loose kernel smut, *S. cruenta* (Kühn) Potter.

Chlamydospore measurements and certain other characters of the material collected in Oklahoma and Kansas indicate that differences between this organism and *Sphacelotheca cruenta* are as wide as those between separate species of some of the other smut fungi, such as the two smuts of oats. Chlamydospores of the Johnson grass smut are larger (Fig. 1), more prominently echinulated, and somewhat darker than those of *S. cruenta*. Microscopic examination also showed that the sporidia and promycelia of the new smut are longer than those of *S. cruenta*. On the other hand, the Johnson grass smut does not differ from *S. cruenta* so sharply as certain physiologic races of *Tilletia tritici*, described by Holton,[4] differ from one another.

Among other interesting characteristics of the Johnson grass smut is its tendency to cause stunting, profuse tillering, and branching of infected plants. The dwarfing often is so severe that smutted panicles are produced on small weak tillers only a few inches tall. It also has been observed that smutted panicles appear much earlier than panicles on noninfected plants. On May 17, 1937, the senior writer observed many smutted panicles of John-

[1] Jackson, H. S. Ustilaginales. *In* Chardon, C. E., and R. A. Toro, Mycological explorations of Venezuela. Puerto Rico, Univ. Monog. Phys. and Biol. Sci. (B) no. 2. 1934.

[2] Johnston, C. O., C. L. Lefebvre, and E. D. Hansing. Kansas mycological notes, 1936. Kansas Acad. Sci. Trans. **40**: [in press.]

[3] Rodenhiser, H. A. Echinulation of chlamydospores and the pathogenicity of a previously undescribed physiologic race of *Sphacelotheca cruenta*. Phytopath. **27**: 643–645. 1937.

[4] Holton, C. S. Studies on seven differentiating characteristics of two physiologic forms of *Tilletia tritici*. Phytopath. **25**: 1091–1098. 1935.

son grass on the roadsides between Ardmore, Oklahoma, and Dallas, Texas, but no noninfected plants were yet in head. The smutted plants were very short, the panicles usually were small, and the sori were characterized by early fragmentation of the peridium and the presence of a long prominent columella.

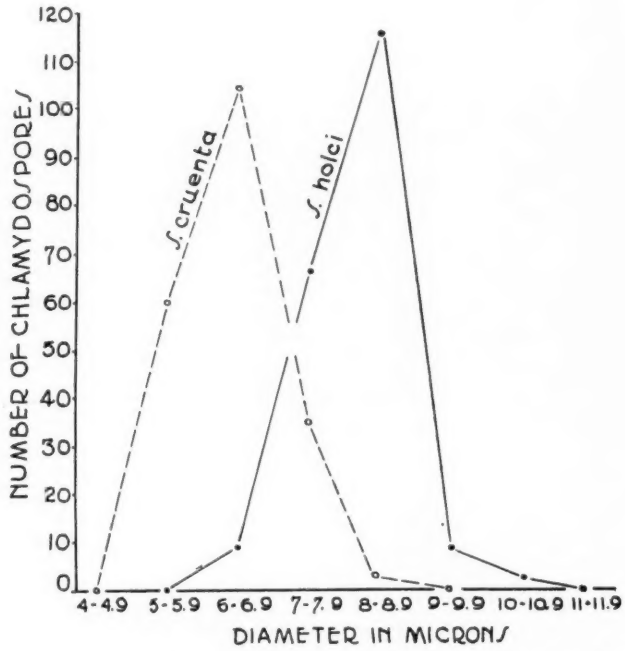

FIG. 1. Comparison of the diameter of 200 chlamydospores each of *Sphacelotheca holci* and *S. cruenta*.

Most of these characteristics also are produced by infections of *Sphacelotheca cruenta* on some varieties of sorghum. This, together with the similarity of the spores, would seem to indicate that the organism is doubtless related to *S. cruenta*. On the other hand, its failure to infect many varieties of cultivated sorghum known to be very susceptible to *S. cruenta* indicates that relationship may be rather distant. It seems, therefore, that further studies, including hybridization experiments, will be necessary to determine the identity of the organism causing loose kernel smut on Johnson grass.—C. O. JOHNSTON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture; C. L. LEFEBVRE, formerly Assistant Professor of Botany, and E. D. HANSING, formerly Graduate Assistant, Dept. of Botany, Kansas Agricultural Experiment Station.

Further Studies on the Parasitism of Rhizoctonia solani on Sugar Beets.[1] —In an earlier paper[2] the writer reported that none of the isolates of *Rhi-*

[1] Cooperative investigations by the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1529 of the Journal Series of the Minnesota Experiment Station.

zoctonia solani Kühn, obtained from sclerotia on potato tubers or lesions on older potato plants, caused rot of older sugar-beet roots. Subsequently, sugar-beet isolates were found to be strongly virulent, as a group, to potatoes. Therefore, it seemed probable that some potato isolates should cause rot of older sugar-beet roots. Since Rhizoctonia is frequently very destructive to potato sprouts in the spring, it was thought that the strongest likelihood of obtaining potato isolates pathogenic to sugar-beet roots would be by isolating from decaying potato sprouts.

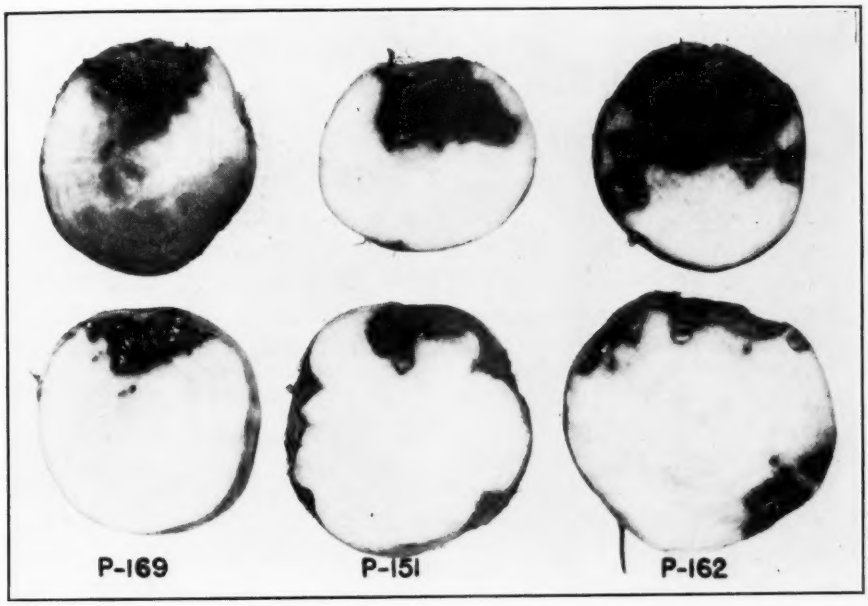

FIG. 1. Cross sections of sugar-beet roots rotted by three potato-sprout isolates of *Rhizoctonia solani*. Two roots rotted by each isolate are shown.

Accordingly, isolations were made from severely injured potato sprouts in the vicinity of St. Paul, Minnesota, and in Louisiana during the springs of 1935 and 1936. Three of these isolates caused appreciable decay of sugar beets (Fig. 1), and 25 additional ones caused smaller amounts of decay. However, 116 isolates from lesions on older potato plants and from sclerotia on tubers were nonpathogenic.

A more detailed account of these investigations will be published later. E. L. LECLERG, University Farm, St. Paul, Minnesota.

Crown Gall on Taxus baccata.—Crown galls have been artificially produced on different species of *Cupressus*,[1] *Libocedrus*,[2] and other conifers[3] of

[2] LeClerg, E. L. Parasitism of *Rhizoctonia solani* on sugar beet. Jour. Agr. Res. [U. S.] 49: 407–431. 1934.

[1] Brown, J. G., and M. M. Evans. Crown gall on a conifer. Phytopath. 23: 97–101. 1933.

[2] Smith, C. O. Crown gall on incense cedar, *Libocedrus decurrens*. Phytopath. 27: 844–849. 1937.

[3] Smith, C. O. Crown gall on conifers. (Abstract.) Phytopath. 25: 894. 1935.

the Pinaceae, but species of the Taxaceae have not been reported as susceptible to crown gall. In table 1 are given the results of puncture inoculations

TABLE 1.—*Summary of results from inoculations on Taxus baccata var. erecta with Pseudomonas tumefaciens*

Inoculation of 1935–1936	Source of culture	Number of inoculation	Number of galls	Diameter in millimeters
January, 1935	*Prunus persica*	15	8	6–11
March	*Prunus persica*	10	7	8–15
May	*Prunus persica*	15	8	5–17
May	Control	5	0	–
June	*Prunus persica*	15	13	4–15
July	*Prunus persica*	5	5	5–13
July	Control	5	0	–
October	*Prunus persica*	5	5	8–10
August, 1936	*Libocedrus decurrens*	5	4	5–7

with *Pseudomonas tumefaciens* on European yew, *Taxus baccata* var. *erecta*. This specimen of *Taxus* was secured from a local nursery and was apparently true to type. It was a shrub 4 feet high and grown in a lathhouse. The branches inoculated were 1 year or more old. Table 1 shows that galls (Fig. 1) were developed by inoculations with cultures isolated from *Prunus*

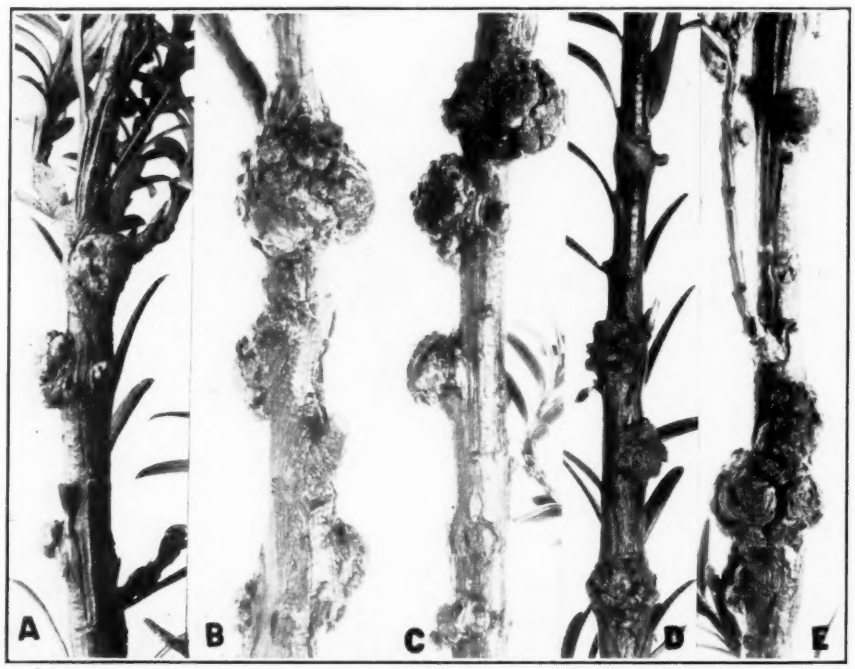

FIG. 1. Artificial galls produced on *Taxus baccata* var. *erecta* (A, B, C, E) by cultures of *Pseudomonas tumefaciens* isolated from *Prunus persica*, and (D), galls by cultures from *Libocedrus decurrens*. A and B represent different ages of the same galls: A, after 8 months and B, after 2 years, 7 months; C, galls photographed after 2½ years; D, galls after 1 year, 3 months; E, galls after 2½ years.

persica and *Libocedrus decurrens*. About 70 per cent of the inoculations gave galls, and these galls are somewhat smaller than those usually produced on other susceptible hosts. Some were formed in nearly every series of inoculations. Under similar conditions, puncture inoculations on *Podocarpus elongata* and *Cephalotaxus fortuni* of the Taxaceae caused no galls.—CLAYTON O. SMITH, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

BOOK REVIEW

LEHMANN, ERNST, HANS KUMMER, and HANS DANNENMANN. *Der Schwarzrost; seine Geschichte, seine Biologie und seine Bekämpfung in Verbindung mit der Berberitzenfrage*. 1st Ed. Pp. xxiv + 584. 87 text illustrations, 1 color plate. J. F. Lehmanns Verlag, München/Berlin. 1937. Price, paper covers 19.50 Rmk., cloth binding 21 Rmk.

With the spread of cereal cultivation to the far places of the earth that accompanied the extensive European migrations of last century, *Puccinia graminis* has assumed a previously unknown economic significance. Gradually a literature commensurate with its new economic importance has grown up in all the principal European languages. From time to time certain phases of the stem-rust problem have been summarized in one or another of these languages, but until the present year no attempt has been made to review thoroughly, in a work exclusively devoted to stem rust, the whole of the vast literature that has grown up around it. In recent years the necessity of such a work has been felt and suggestions have not been wanting that it should be undertaken. The appearance of *Der Schwarzrost* should therefore be hailed with satisfaction by all those connected in any way with stem-rust investigations.

The work has been undertaken in no haphazard manner. The thoroughness with which the literature has been reviewed is attested by the 57-page bibliography containing references to approximately 2000 publications. The arrangement of the material gives evidence that the stem-rust problem was carefully considered from its various angles before the work was undertaken. The length of the book is due largely to the anxiety of the authors that no phase of the problem should escape adequate consideration.

The book commences with a historical survey of stem-rust investigations, a survey that is to a certain extent a summary of the book as well. In this survey, as, indeed, in the book as a whole, special attention is devoted to the relationship between stem rust and the barberry. After this introductory survey the main body of the book falls into five sections; the amount of space allotted to each gives an idea of where the authors place their main emphasis: 1. The barberry (74 pages). 2. The biology of stem-rust parasitism —on both the barberry and the graminaceous hosts (228 pages). 3. Epidemiology (25 pages). 4. Distribution of stem rust and damages caused by it (40 pages). 5. The control of stem rust—with special stress placed on barberry eradication (105 pages).

The special prominence given the barberry results from the fact that recent investigations have shown that it still plays a significant part in stem-rust outbreaks in Germany. The whole question of its relationship to stem rust has, therefore, been treated with exceptional thoroughness. A detailed account is given of the distribution of the genus *Berberis*, particular emphasis being placed on the common barberry, its origin, distribution throughout the world, and the history of how it came to be associated with stem rust on cereals. In this connection it is interesting to note how the great epidemic in Europe in 1804 helped to establish in the minds of Banks, Windt, and others, the certainty of the relation of the rust on barberry to stem rust on cereals, although the actual proof was delayed for more than half a century. The consequences of de Bary's work are dealt with at length and a detailed history is given of the anti-barberry legislation enacted up to the present in different parts of the world. The extensive literature on the relation of the barberry to stem rust in North America is thoroughly reviewed and a full account is given of the barberry-eradication campaign in the United States and its consequences. In the discussion of the host-parasite relations of barberry and stem rust the views of Allen and Andrus on the mode of union of pycniospores and haploid hyphae are stated but no mention is made of Craigie's conception, although his report on the union of pycniospores and haploid hyphae in *P. helianthi* is cited in the bibliography.

Although much space is devoted to the barberry the longest and most important sections of the book deal with the relation of stem rust to its graminaceous hosts. The section dealing with physiologic specialization is, in fact, a complete manual or guide to the culturing of rust in the greenhouse and the determination of physiologic races of the wheat, oat, and rye varieties of stem rust. Every phase of the host-parasite relationship is considered: the entrance of the organism into and its development within the host, resistance and susceptibility of the host at various stages of growth, the influence of diverse environmental factors on the expression of the disease, and many others.

Perhaps the most striking feature of the book at first glance is the highly organized arrangement of the material, particularly in the parts dealing with the biology of parasitism. Here the phase of the rust on the grasses and that on barberry are considered separately. Following the practice of Fischer and Gäumann in their *Biologie der pflanzenbewohnenden parasitischen Pilze* the authors conduct the discussion under two main headings (1) the conditions necessary for the establishment of parasitic relations, and (2) the course of parasitic relations. The first of these headings is discussed separately from the point of view of both parasite and host. It thus becomes necessary to view certain factors first from the standpoint of the diploid phase of the rust, secondly from the standpoint of the grass host, thirdly from the point of view of the haploid phase of the rust and fourthly from the point of view of the barberry host. Accordingly, factors such as light, temperature, moisture, and so forth are sometimes considered from two or more of these viewpoints. Similarly, the infection types on cereals must, for the sake of the argument, be dealt with to a certain extent from the standpoint of the host and again, later, from that of the parasite. This natural and logical division of material leads inevitably to a considerable complexity of organization, and owing to the fact that these conditions are in many cases the same for both host and parasite a certain amount of repetition becomes unavoidable. Undue repetition is, however, skilfully avoided by the authors wherever possible. While this type of organization contributes much to the thoroughness of the discussion of the stem-rust problem, it also adds considerably to the length of the book.

In so comprehensive a survey occasional errors would be expected. For example, on p. 222 Newton, Johnson, and Brown are erroneously given credit for isolating a race of *P. graminis secalis* from a cross between the wheat and rye varieties of stem rust, and, on p. 178, a paper by W. L. Gordon is mistakenly cited in relation to the effect of carbon-dioxide concentration on rust development.

The book is adequately illustrated and clearly printed on paper of good quality, but the binding is scarcely strong enough for so bulky a volume.

The extreme thoroughness with which this compilation has been carried out makes it probable that it will serve as a basis for similar works in other languages. While it is regrettable that no such work is available in English, its existence in German is at least a partial compensation.—T. JOHNSON, Dominion Rust Research Laboratory, Winnipeg, Manitoba.